PROTEIN METABOLISM

R. B. FISHER

METHUEN'S MONOGRAPHS ON BIOCHEMICAL SUBJECTS

Protein Metabolism

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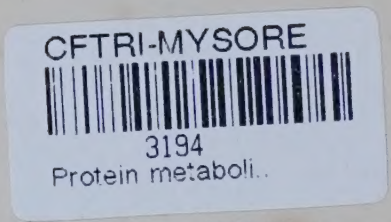

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PREFACE

A book of this small size, concerned to cover so large a topic, can touch the topic only at points. If the book is to have any value, these points must group around some significant trend in ideas. The theme around which this book has been built is that protein metabolism is concerned with the concerted metabolism of the amino-acids, a process regarded as something distinct from the metabolism of the individual amino-acids.

In pursuit of this theme many topics of great intrinsic interest have been left on one side. The transformations of individual amino-acids in the body are naturally little touched on, and in correspondence with this omission such topics as the synthesis and catabolism of purines, pyrimidines, porphyrins, creatine and other non-protein nitrogenous compounds are also not discussed. Where other interesting and important topics have been left out, as for instance the specific dynamic action of proteins, the criterion has been that the omitted topics could not be brought to bear on the thesis of the book in the space that could be afforded to them.

This insistence on thesis may earn disapproval. But there is practical justification for it. The bibliographies of the reviews of the metabolism of proteins and amino-acids which have been published in the *Annual Reviews of Biochemistry* in the years 1932–52 would in themselves occupy more space than the whole of this book, and there was already enough substance in protein metabolism before the beginning of the publication of the *Annual Reviews* (in 1932) to form the basis for the admirable monograph by Mitchell and Hamilton (1929), which is in itself much larger than this book. It follows

that a rigorous selection of some sort is inevitable. It seems best that this selection should seek to establish some basic theme, since it may be predicted with confidence that the number of papers noticed in the next *Annual Review of Biochemistry* will be little less than the total bibliography of this book, and that as a consequence much of what appears in detail here may well be superseded on the day of publication.

There is also the justification for the approach selected that it places emphasis on the structure of scientific advance. It is a matter of importance that what is newer in the way of scientific conclusions can only be better if the interpretations placed on the newer facts are based on sound interpretations of earlier facts. It is not always realized that interpretations are based more often on considerations of relative plausibility than of logical necessity, and that what constitutes relative plausibility is to a large extent a matter of current fashion in abstraction.

It is hoped that the approach used to the problems of protein metabolism may persuade some readers of the value of these considerations, and may show why it is inevitable that what appears in the literature as unequivocally established may not always be so, so that more extensive and critical examination of older literature may prove of more value to the student than some of the effort which is customarily devoted to the acquisition of up-to-the-minute knowledge of the recent literature.

It has been a matter of deliberate policy in this book to reduce references to the very recent literature to the minimum, largely for the reasons already discussed. There is the additional argument in favour of this policy that the natural inclination to suppose that the latest is the best tends to induce some disproportion in the thinking of both writer and reader.

CONTENTS

To

L.M.V.S.F. and J.S.F.

with many thanks

This book owes its inception to Professor Sir R. A. Peters, F.R.S. Any virtues it possesses are the outcome of his teaching, his encouragement and his critical comments.

I am much indebted to Miss Sylvia Smith for invaluable assistance in all stages of the production of the book.

R.B.F.

CHAPTER I

DIGESTION AND ABSORPTION OF PROTEIN

The first step in an inquiry into the nature of the chemical processes of protein metabolism should be to determine the form in which protein enters the body, that is to say the form in which it enters the tissue fluids lying below the intestinal mucosal membrane. So long as they lie in the lumen of the intestine foodstuffs which have been taken in by mouth have to be considered physiologically as outside the body.

Processes of protein digestion:

The inquiry into protein metabolism therefore starts with a study of protein digestion. Protein digestion starts normally in the stomach. The gastric mucosa secretes an enzyme-precursor pepsinogen which is transformed by the acid secretion of the parietal cells of the mucosa into pepsin, an endopeptidase in Bergmann's terminology (Bergmann and Fruton, 1937). The mode of formation of the enzyme suggests that the likelihood of conversion of pepsinogen to pepsin will be less the more efficiently the secretions are mixed with the food: the food proteins buffer the acid which is needed to activate the pepsinogen. A lengthy controversy concerning the reality of a second gastric protease appears to have been resolved recently, and it may now be taken that a distinct second protease, rennin, can be obtained from gastric mucosa (Berridge, 1945). It is not certain that rennin is secreted into the gastric juice in any circumstances.

The pancreatic secretion which is poured into the

duodenum contains a mixture of at least two endopeptidases, trypsin and chymotrypsin, and at least one exopeptidase, carboxypeptidase. The succus entericus, the secretion of the cells of the mucosa of the small intestine, is said to contain a series of exopeptidases which were collectively known as erepsin. For present purposes there is no need to distinguish between the component enzymes of these different physiological secretions.

Protein digestion appears at first sight to be the resultant of successive action of gastric, pancreatic and ereptic secretions on the foodstuff. In all probability it is essentially much simpler. Peters and Van Slyke (1946) review evidence indicating that in man the removal of the whole or the greater part of the stomach is without effect on the capacity of the subject to assimilate protein, so we may suppose that whatever digestion occurs in

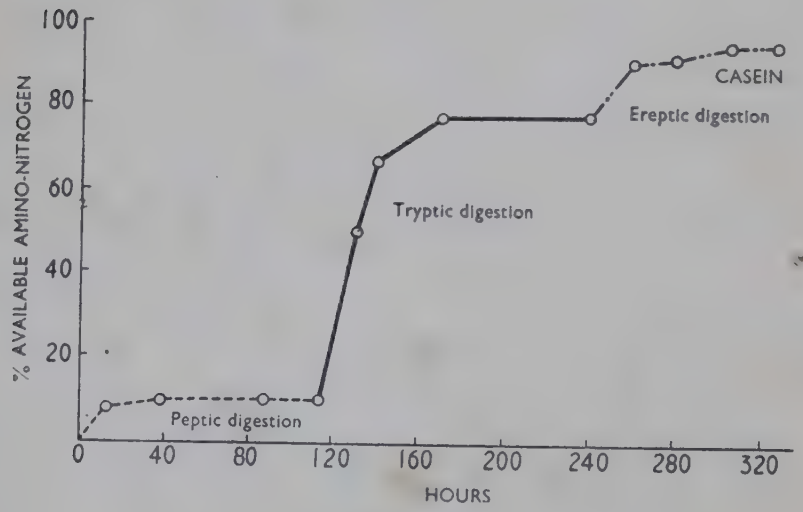


FIG. 1

The time-course of the digestion of casein by the successive action of pepsin, trypsin and erepsin. (*Redrawn from the data of Dunn and Lewis* (1921), J. Biol. Chem., **49**, 343.)

the stomach is not essential. Florey, Wright and Jennings (1941) showed that the weight of the evidence is that the peptidase activity of succus entericus is due to mucosal cells suspended in it. Peptidase activity in the lumen of the intestine would appear to be due to shedding of mucosa, not to secretion by the mucosa. There is no ground for supposing that shedding of mucosa occurs more readily during than between periods of digestive activity. It is probable therefore that the numerous studies of 'erepsin' which have concerned themselves with the study of extracts of intestinal mucosa have little bearing on the digestive activity of the small intestine.

The rate of protein digestion:

Figs. 1 and 2 based on the work of Dunn and Lewis (1921) and of Frankel (1916) respectively, serve to illustrate the time-course of proteolysis, when the three possible types of digestive secretion are permitted to act in succession, optimal conditions for each being set up in turn. From the second of these figures estimates of the times taken for 50 per cent. and 90 per cent. completion of each stage have been computed. Table 1 lists the times estimated to be required for completion of each stage of digestion on the alternative assumptions of completion at the average rate observed during (*a*) the first 50 per cent., (*b*) the first 90 per cent. of the digestion achieved by that particular enzyme. (See page 5.)

Two points deserve special note in this Table. First, estimates of digestion times for widely different proteins are similar although they have been obtained by different workers using different sources and almost certainly different concentrations of enzymes. Second, even on the most generous assumptions, the time-course of liberation of amino-N is too slow to fit with the view that proteins must be digested to amino-acids before they are absorbed. Even if it be supposed that neither

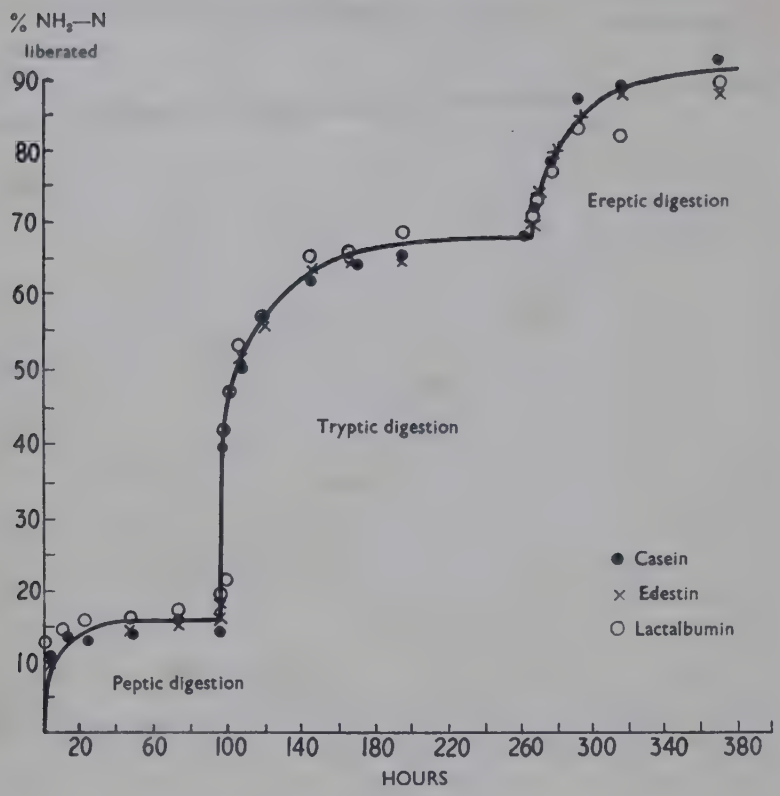


FIG. 2

The time-course of the digestion of various proteins by the successive action of pepsin, trypsin and erepsin. (*Redrawn from the data of Frankel* (1916).)

peptic nor ereptic digestion has any physiological significance, so that the time-course of tryptic digestion represents the physiological time-course of proteolysis, the time taken for complete hydrolysis of protein has to be estimated as of the order of twenty-four hours on the most generous basis.

This is difficult to reconcile with such observations as those of Janney (1915) on the time-course of the excretion of extra glucose and of extra nitrogen following the

TABLE 1

Approximate estimates of the time necessary for the digestion of protein in the alimentary tract, based on the data presented in Fig. 1

Enzyme	*Time for 50% completion of digestion*	*Time for complete digestion at this rate*	*Time for 90% completion of digestion*	*Time for complete digestion at this rate*
Pepsin	2 hrs.	4 hrs.	40 hrs.	45 hrs.
Trypsin	4	8	50	55
Erepsin	12	24	50	55
Sum .	—	**36 hrs.**	—	**155 hrs.**

Each enzyme is supposed to produce its maximal action before the next enzyme in sequence begins to act.

administration on separate occasions of glucose, protein and an acid hydrolysate of protein to phlorhizinized dogs. Typical findings are illustrated in Fig. 3. It is difficult to see how these findings could be accounted for unless protein absorption is as rapid as that of a mixture of amino-acids. This means either that proteins do not require to be digested to amino-acids in order to be absorbed or that the *in vitro* evidence of Dunn and Lewis and of Frankel and of many other investigators underestimates the potentiality of the body for proteolysis by a factor of twenty or thirty. At present the question cannot be settled. It remains to be pointed out that the existing evidence is certainly insufficient to support the current view that complete proteolysis precedes absorption.

The nature of the products of absorption:

The view that proteins are broken down in the intestine to amino-acids and are absorbed as such is not solely

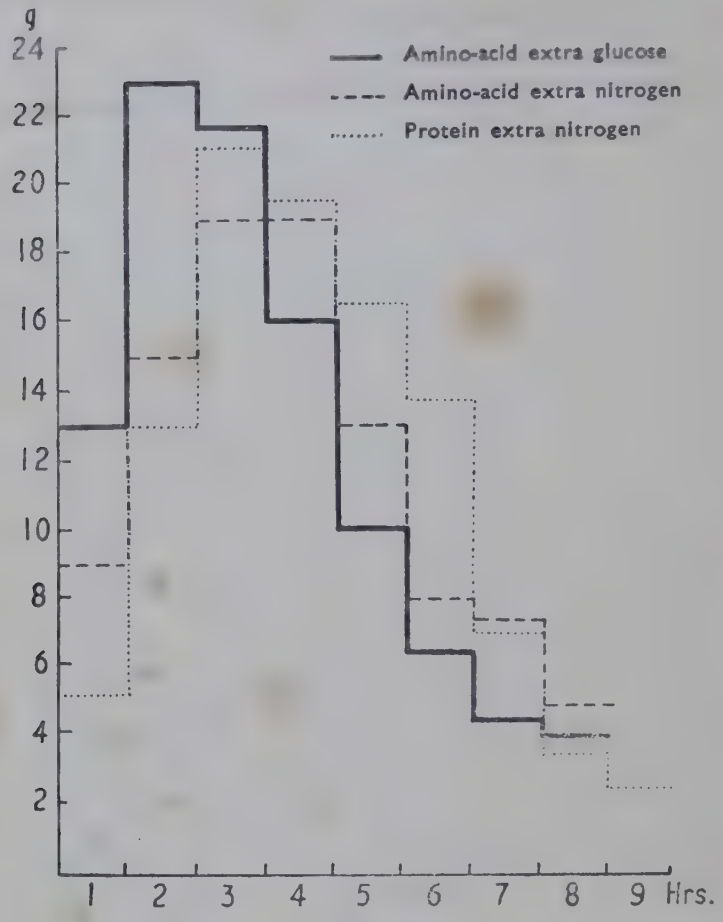


FIG. 3

The course of excretion of extra nitrogen by a phlorrhizinized dog after administration of protein or of a protein hydrolysate by mouth. The course of excretion of extra glucose after giving the hydrolysate is also shown. (*Redrawn from the data of Janney*, 1915.)

dependent on a demonstration of the capacity of the digestive secretions to hydrolyse protein completely. In the early part of this century it was believed that

protein must be synthesized in the wall of the intestine, and that it was passed into the bloodstream as such. Folin and Denis (1912) first showed quite clearly that the non-protein nitrogen of blood rises during protein absorption. Immediately afterwards Van Slyke and Meyer (1912), using a procedure which set a new standard of delicacy in analytical methods, showed that there was a rise in α-amino-N in blood during protein absorption. This rise was interpreted to mean that α-amino-acids were passed into the blood-stream during protein absorption. The analytical method is now known to estimate the free amino-groups of peptides and other free amino-groups.

Very shortly afterwards Abel, Rowntree and Turner (1913–14) contributed to this topic. Abel had been investigating the possibility of supplementing deficient renal function in patients with renal disease by passing blood from an artery through collodion tubes immersed in saline, and back into a vein. Experiments to test this procedure of vividialysis were made on dogs, and some 300 litres of dialysate were collected. There is no indication that any of the dogs were absorbing protein at the time of the experiments. Yet the fact that Abel, Rowntree and Turner found amino-acids in their dialysates has been taken as evidence of absorption of protein principally as amino-acids. After removing basic amino-acids (and presumably basic peptides), Abel's dialysates contained 1·5 g. amino-N, as estimated by the method of Van Slyke and Meyer. Only 0·055 g. of this was isolated in the form of two amino-acids, alanine (containing 0·045 g. amino-N) and valine (0·01 g. amino-N). It would not in any event have been surprising if the amino-acids present in the dialysates had not been recovered quantitatively. But it is very difficult to regard an investigation not designed to study protein absorption, and carried out on animals not absorbing protein, as a chief prop to the view that protein

is absorbed in the form of amino-acids, especially as not more than 4 per cent. of the material investigated which was designated by implication as amino-acid was actually shown to be amino-acid. There is no indication that Abel and his colleagues agreed to some of the interpretations that have been put on their work. Discussion of it is necessary here because it is still possible for a well-known text-book to view it in the following light:

> '. . . there is no *a priori* reason to believe that the blood carries the proteins from the alimentary tract in any other form than that of the amino-acids. Strongly in support of this conclusion is that after a protein meal there is an increase in the non-protein nitrogen . . . of the blood, and particularly in the portal blood. The final proof was rendered possible by van Slyke's method for the detection of small quantities of amino-acids in the blood and tissues, and ultimately by the actual isolation of amino-acids from the dialysate prepared by circulating blood from a living animal through a collodion dialyser.' (LOVATT EVANS: 9th ed., p. 880.)

The conjunction of the uncertainty concerning the possibility of complete digestion of protein in the time available with the uncertainty of the evidence for amino-acids as the sole material added to the blood-stream during absorption renders 'proof' an inappropriate word in this connection at this time.

The interpretation of changes in blood composition during protein absorption:

In metabolic investigations it is easy to lay disproportionate stress on changes in concentration of substances in the blood. If there is good reason to suppose that a substance of a particular class is being added to the blood at a particular time, and if the concentration in the blood of this class of substance rises at this time, the rise may be interpreted as a consequence of the known process of

addition. But the absence of a detectable rise in such circumstances could not be taken as meaning that there was absence of addition. When King and Rapport (1933) administered as much as 5 g. of tyrosine to a dog by intravenous injection, little or none of the tyrosine could be found in the blood five minutes later, in any of the forms of amino-nitrogen, phenol, non-protein-nitrogen or urea. In order to obtain this amount of tyrosine from a protein meal it would be necessary to absorb the products of digestion of 100–240 g. of protein.

A change in concentration in the blood merely signalizes a change in relation between the totality of processes adding the substance to the blood and the totality of processes removing it. Thus, for instance, if substances A and B are being added to the blood at equal rates, their relative concentrations in the blood will depend upon the respective relations between blood concentrations and rates of removal. If A is more rapidly removed from the blood than is B when the concentrations are equal, the concentration of A in the blood will be lower than that of B when both are being added at equal rates.

In general, blood concentrations cannot be interpreted as indicative of the rate of either additive or subtractive processes unless there is good independent evidence of the bearing of the other of the two processes on blood concentration. In some simple systems the interpretation can be made almost unequivocally, as when simultaneous arterial and portal blood samples are taken during intestinal absorption of protein. Here the blood passes through one tissue only, but it is still necessary to assume (*a*) that there is no abstraction of non-protein-N by the tissues of the intestine and (*b*) that the intestinal lymph carries off no non-protein-N, if the rise in non-protein-N content of the issuing blood is to be taken as a good indication of the process of addition.

On this basis, London and Kotschneff's (1934) observations on the arterio-portal concentration differences in amino-N do give unequivocal evidence of the addition of amino-N to the blood during protein absorption, though they may under-estimate the rate of absorption. This work was done using London's angiostomy technique, which permits the withdrawal from the conscious animal of samples from any previously prepared artery or vein. Christensen and Lynch (1946) have criticized the analytical methods used by London and Kotschneff, so that it is still not clear whether one should accept the claim of these workers that two-thirds of the non-protein amino-N added to the blood during passage through the tissues of the wall of a small intestine absorbing protein is peptide nitrogen.

There is evidence that the lining of the gastro-intestinal tract is pervious to protein derivatives of a higher order of complexity than simple amino-acids. Immunological evidence of the absorption from the gastro-intestinal tract of protein fragments large enough to exhibit the specificity of the parent protein has been obtained many times. Unfortunately, it is not known where in the alimentary tract this absorption takes place. Two instances of interest are that Barnes and Bueno (1933) produced anaphylaxis in guinea-pigs by feeding thyreoglobulin to animals sensitized to it, and that Alexander, Shirley and Allen (1936) found a substance reacting with a specific antibody in thoracic-duct lymph after feeding egg-white to dogs.

The path of absorption of protein:

Alexander *et al.* (loc. cit.) failed to find evidence of absorption of egg-white into portal blood although it or something having its immunological properties is readily demonstrated in thoracic duct lymph. This might mean no more than that the absorption does not take place in the part of the alimentary tract drained by the portal

vein, but it would not be a surprising finding even if the absorption took place in the small intestine.

Products of absorption must be supposed to pass from the mucosal cells into the tissue fluid of the submucosal space. In this space there lie the blood capillaries, very close to the mucosal cells, and the lymphatic vessel or vessels. Substances passed into the tissue fluid must fall into categories of possessing greater or less ability to permeate the wall of the capillary vessels. It seems doubtful that any of these substances will have difficulty in permeating the lymphatic wall. If we consider what is likely to happen to a simple molecule like glucose in such a system it may prove helpful. The absorbed glucose will appear in relatively high concentration at the periphery of the villus in that part of the tissue fluid in which the capillary blood vessels lie, and will disturb the osmotic balance between blood plasma and tissue fluid. Diffusion of water out of the capillary and of glucose into it will tend to redress the balance. But even if the capillary wall offers no hindrance to diffusion of glucose, the balance will be redressed more by the diffusion of water out than by the diffusion of glucose into the capillary: water has the higher diffusion coefficient. Thus we may expect that the hydrostatic pressure in the villus will rise and that lymph flow will increase. Even in this instance in which access to the capillary blood of the substance being absorbed is maximal, it seems probable that a significant fraction will be swept on a stream of tissue fluid into the lymphatics and will first enter the blood circulation in the neck. It will naturally follow that any absorbed substance which experiences significant difficulty in crossing the capillary membrane, whether due to molecular size or chemical constitution, may be expected to enter the circulation preferentially by way of the lymphatics. It is for this reason that the absence of egg-white antigen in portal blood during absorption of egg-white is not surprising.

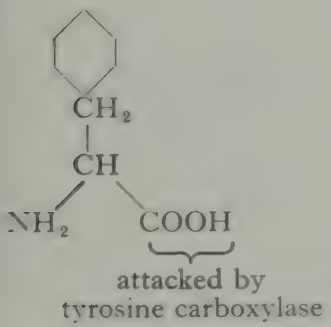
It also follows from these considerations that if protein were absorbed from the intestine in the form of a mixture of peptides and amino-acids, the components of higher molecular weight would tend to pass into the lymph and those of lower molecular weight into the blood, so that the question of the forms in which protein enters the body fluids cannot be answered in terms of portal blood concentrations or even of arterio-portal concentration differences.

Thus, although no direct evidence for it can be adduced, the view that proteins may be absorbed into the body largely in the form of split-products of higher order of complexity than amino-acids still requires to be taken into account. There is no evidence that protein can be digested to amino-acids sufficiently rapidly to permit of absorption in this form. The experiments adduced in support of the amino-acid hypothesis give only a partial picture: they show that amino-acid accumulates in the blood in these circumstances but they do not provide the evidence necessary to show that this is a major form of absorption product.

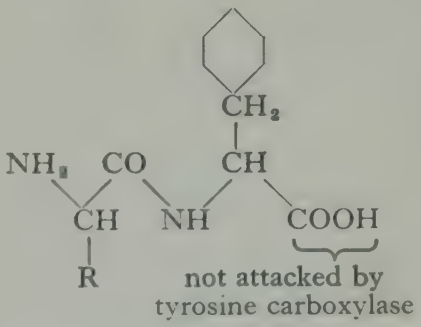
Dent and Schilling (1949) have reported experiments on dogs in which portal and jugular samples were examined by paper partition chromatography. The amino-N of ultra-filtrates of plasma was also determined in some of these experiments (addendum to the above paper by Christensen). Peptides were not detected in portal blood by partition chromatography. This work might have contributed more to the solution of the problem if arterio-portal concentration differences had been investigated: the jugular-portal differences shown to occur in some of the experiments do not contribute much useful evidence. Unfortunately the dogs had been subjected to partial gastrectomy, which, apart from causing loss of weight, appears to change the concentration of amino-acids in the blood, so that Christensen's figures for amino-N obtained by the much more specific

ninhydrin method of Hamilton and Van Slyke (1943), which measures the amino-N adjacent to a free carboxyl, i.e. that of free amino-acids, cannot be compared with older figures for dogs, obtained by the original nitrous acid method.

Metabolic differences between amino-acids and peptides: The topic of the form in which protein is absorbed from the intestine is fundamental to the whole problem of the intermediary metabolism of protein. Although very little is known of the metabolism of peptides, what little is known suggests that they may be treated by the body very differently from free amino-acids. For instance, Zeller's (1948) 'ophio-amino-acid oxidase', a preparation from snake venom, readily oxidizes free L-α-amino-acids, but is unable to oxidize many peptides with free amino-groups. Tyroxine decarboxylase will not attack dipep-



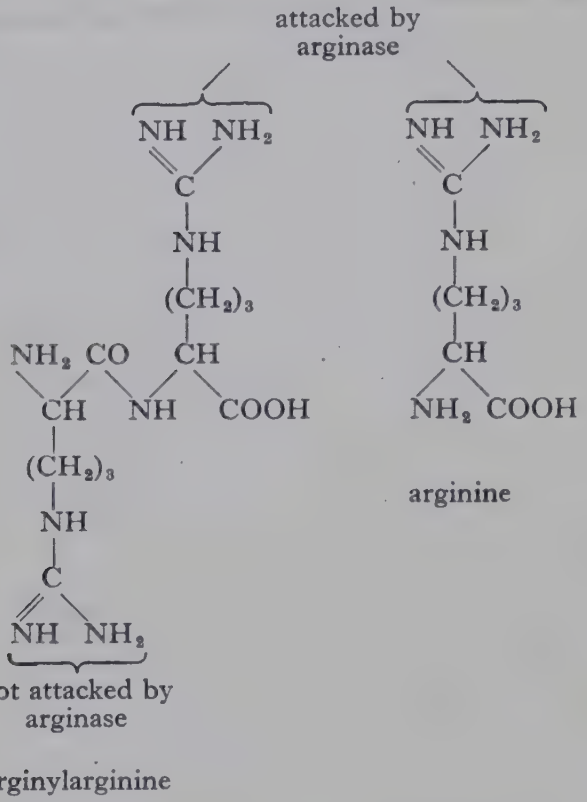
tyrosine



R-yl-tyrosine

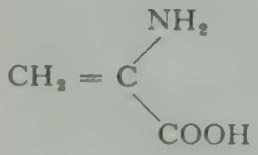
tides in which the tyrosine carboxyl is free (Zamecnik and Stephenson, 1947), and arginase will apparently split only that guanidine group belonging to an arginine residue with a free carboxyl: the protamine clupein, which contains over 80 per cent. of arginine, is not attacked by arginase (Kossel and Dakin, 1904), and only one of the guanidine groups of arginyl-arginine is

hydrolysed (Edlbacher and Bonem, 1925; Edlbacher and Burchard, 1931).



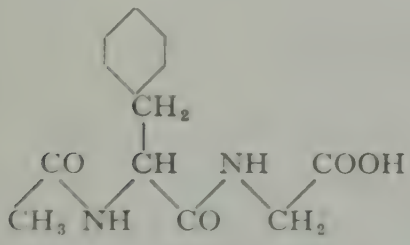
These are all instances of enzymes capable of transforming amino-acids which cannot transform peptides. No instance is known of an enzyme capable of transforming an amino-acid when in peptide linkage but not when it is free, unless it be possible to consider the action of dehydropeptidases in this category (Greenstein, 1948). These enzymes liberate ammonia and pyruvic acid from derivatives of dehydroalanine, amino-acrylic acid. However, since biochemists rarely discover enzymes that have not been sought specifically, this does

not necessarily mean more than that serious thought has not so far often been given to the possibility that many of the catabolic changes to which amino-acids are subject in the body may occur whilst they are conjugated with other amino-acids.

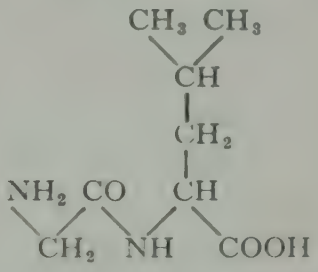


amino-acrylic acid

One interesting instance of the effect of conjugation on the fate of a compound is afforded by the action of cysteine-activated papain, an endopeptidase, on the peptide glycyl-L-leucine. Bergmann and Fruton (1941) showed the enzyme to have no effect on this peptide or on acetyl-L-phenylanalylglycine. But when both these



acetyl-phenylalanyl-glycine



glycyl-leucine

peptides were present in the reaction mixture hydrolysis of the glycyl-leucine appeared to occur. This was shown to be a consequence of the condensation of the two peptides, the enzyme then being capable of splitting the terminal leucine and glycine residues successively from this product, leaving again the resistant acetylphenyl-alanylglycine. In this instance the transformation

effected with the aid of the second peptide is a mere hydrolysis into amino-acids, but there is no reason why some enzymes should not require similar 'co-peptides' in order to effect transformation other than simple hydrolysis.

It is possible that side-chain transformations can be effected in peptides without scission of the peptide. Sizer (1947) has claimed to have established that mushroom tyrosinase can oxidize the tyrosyl groups of purified proteins. If, however, such side-chain reactions were common in proteins, as distinct from small peptides, the modified amino-acid residues formed might be expected to have been demonstrated in native proteins.

Since condensation with one another into peptides seems to render amino-acids resistant to the biological agencies capable of transforming them when free, peptides must presumably either be hydrolysed or be metabolized by pathways other than those open to the free amino-acids. It is therefore open to doubt whether the known processes of transformation of amino-acids can be taken as reliable guides to the paths of protein metabolism.

The meaning of 'protein absorption':

It is tacitly assumed in discussion of protein absorption that the products of digestion of protein diffuse through the mucosa of the intestine, so that knowledge of what leaves the lumen constitutes knowledge of what appears in the tissue fluids of the body. But we shall see that much evidence shows that labelled amino-acids introduced into the body by any route make their appearance in the proteins of the intestinal wall, particularly the mucosa, more promptly than in any other major tissue of the body. These labelled constituents also disappear from intestinal protein with unusual celerity.

Thus during absorption the amount of intestinal mucosal protein is increasing. In the post-absorptive

state it is diminishing, and it seems reasonable to suppose that the products of catabolism of intestinal protein, which may indeed consist entirely of amino-acids, but are more likely to be made up in large part of specific peptides, constitute a large part of what is added to the tissue fluids during protein absorption. Naturally, where a food protein deviates largely from the pattern of amino-acids required to synthesize intestinal protein, residues are bound to be left over from the process of protein synthesis and these may well appear in the tissue fluids as free amino-acids. The finding by Dent and Schilling (loc. cit.) that there was little or no rise in plasma amino-acid concentration during absorption of homologous serum albumin is quite consonant with this view. Other evidence pointing in the same general direction is reviewed in later chapters.

In this we are completing the circle to the point of view current before Van Slyke and Meyer's work in 1912, though with a difference. It was then thought that the first step in protein absorption was the synthesis of peptides or proteins in the intestinal mucosa, but these were then thought to pass into the blood. Both these views might be thought difficult to sustain in face of evidence that animals can be maintained in excellent health on a diet in which all protein is replaced by a mixture of purified amino-acids given either by mouth or by vein. The evidence afforded by oral administration is not particularly strong: there is no particular reason why protein synthesis in the mucosa should not start with a mixture of amino-acids. The evidence that protein requirements can be satisfied by intravenous amino-acids is no stronger: as we shall see later it is as easy to meet the protein requirements of a dog by giving homologous plasma protein intravenously as it is to do so by giving purified amino-acids. If the one piece of evidence is taken to suggest that the normal form in which protein circulates in the body is that of free

amino-acids, the other speaks equally strongly for whole protein molecules as the form of currency. Neither therefore carries any weight in this respect. Whatever the normal form of currency in protein metabolism, it is equally formed from a mixture of amino-acids and a mixture of homologous plasma proteins.

Conclusion:

There is little evidence for the necessity for peptic digestion and little certainty of the occurrence of 'ereptic' digestion of proteins, but tryptic digestion seems essential to normal protein absorption. The evidence that the products of the digestion are amino-acids and that protein passes into the blood-stream in this form is incomplete, and is at variance with the time taken for complete digestion of protein. Evidence for the chemical form of the products of protein absorption is unreliable.

The problem of the form of the normal products of absorption is important, because it determines the course of investigation into the details of protein metabolism. It is suggested that there is enough evidence to consider seriously the possibility that peptides rather than amino-acids are the normal currency of protein metabolism.

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CHAPTER II

THE OVERALL PICTURE OF PROTEIN METABOLISM

The broad outlines of protein metabolism are simple so long as attention is confined to the fate of its characteristic constituent, nitrogen. In a fully-grown animal, subsisting on a diet which provides adequate energy and a constant daily intake of nitrogen, the mean nitrogen excretion *per diem* is equal to the mean nitrogen intake. In these circumstances a state of nitrogenous equilibrium is said to exist. Exact balances are difficult to strike and require elaborate investigations. Ingestion and absorption of food is periodic, not continuous, in most experimental animals, and there is a marked time-lag between ingestion and excretion (see Fig. 3, Chap. I). Losses of nitrogen also occur by several paths, by loss of urine, faeces and sweat and by attrition of the skin and its appendages. The urine is by far the most important channel of loss, and losses by other channels are little affected by change in nitrogen intake, so that little error is usually introduced by interpreting changes in urinary excretion as indicators of change in total loss of nitrogen, provided that the changes are averaged over sufficiently long periods of time.

Exogenous and endogenous nitrogen metabolism:

In the mammalia the greater part of urinary nitrogen is present as urea. When a mammalian subject passes from nitrogenous equilibrium on a high-protein diet to nitrogenous equilibrium on a low-protein diet it is the amount of urea excreted which changes most profoundly.

The nature of the change in urinary nitrogen partition in man is illustrated, in round figures, in Table 2. It

TABLE 2

An approximate representation of the effect of change from equilibrium on high- to equilibrium on low-nitrogen intake on the partition of nitrogen in the urine of an adult human subject

Form of excretion of nitrogen	*Daily urinary nitrogen excretion of subject in nitrogen equilibrium*	
	High nitrogen diet	*Low nitrogen diet*
Total nitrogen . .	15 g.	5 g.
Urea . . .	12 g.	3 g.
Creatinine . .	½ g.	½ g.
Ammonia . . .	½ g.	½ g.
Uric acid . . .	1 g.	½ g.
Undetermined . .	1 g.	½ g.

was this type of observation that led Folin (1905) and Folin and Denis (1912) to postulate that there were two series of processes involved in protein metabolism, which they distinguished as exogenous and endogenous. The exogenous processes were taken to be those by which incoming food-stuffs were directly catabolized at rates determined by rates of supply. Endogenous processes were taken to be those whose rates were determined primarily by the activities of different physiological processes and only secondarily, if at all, by the rate of supply of nitrogen. The excretion of creatinine and of uric acid provide good examples of this type of process. The relative constancy of creatinine excretion has been confirmed by many workers since Folin, and it appears

that uric acid excretion shows similar constancy provided that the diet is free from purines.

Endogenous nitrogen metabolism and erythropoiesis: Recent work on the life of the erythrocyte provides an excellent example of a process contributory to endogenous nitrogen metabolism. The fact that the number of erythrocytes per unit volume of blood remains almost constant indicates that the time-course of formation of erythrocytes is essentially similar to that of their destruction. Thus, the demonstration by three different methods that each human erythrocyte survives in the circulating blood for a period of the order of 120 days can be taken to mean that approximately $\frac{1}{120}$th of the erythrocytes are replaced each day. The establishment of this figure is due to Callender, Powell and Witts (1945), Jope (1946), Shemin and Rittenberg (1946).

Since each transformation of a haemocytoblast into an erythrocyte involves the extrusion of a purine-rich nucleus, it is to be supposed that the constant rate of erythropoiesis implies a constant rate of purine catabolism, which ought to be reflected in a constant contribution to uric acid excretion. Riddle (1930) showed that the administration of liver to patients with pernicious anaemia produced a striking rise in uric acid excretion, occurring over the period occupied by the reticulocyte peak. The administration of similar amounts of liver to normal subjects did not affect uric acid excretion. These phenomena are illustrated in Figs. 4 and 5.

Krafka (1929, 1930) produced increased erythropoiesis in Dalmatian dogs by bleeding or administration of phenyl-hydrazine, the animals being kept on purine-free diets. Both procedures were followed by rise in uric acid excretion, contemporaneous with the reticulocyte peak.

There seems therefore good ground to suppose that erythropoiesis contributes significantly to the endogenous

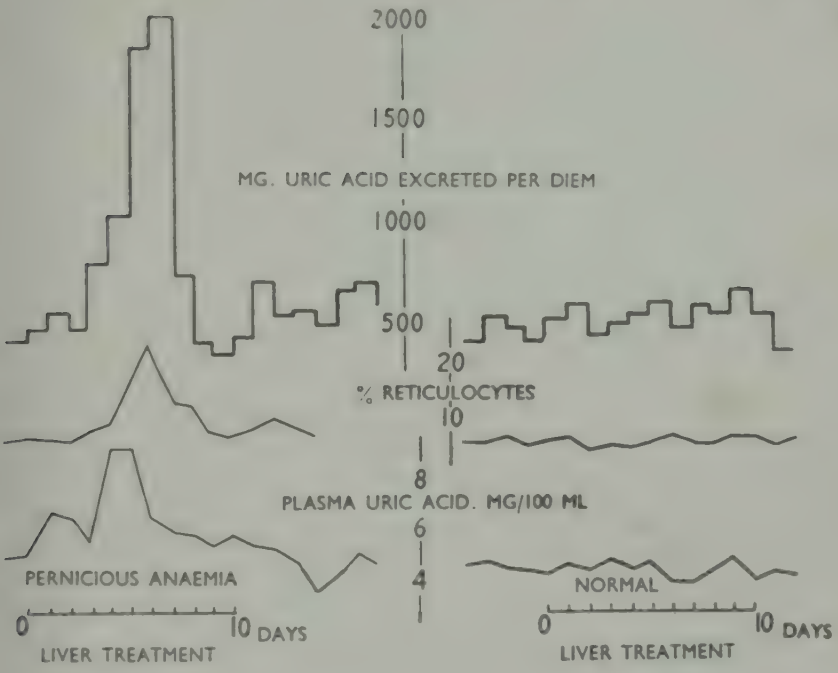

FIG. 4 FIG. 5

The effects of administration of liver extract on the blood uric acid concentration, the rate of uric acid excretion in the urine, and the percentage of erythrocytes showing reticulation in a subject suffering from pernicious anaemia (Fig. 4) and in a normal subject (Fig. 5). (*Redrawn from Riddle*, 1930.)

purine metabolism of the body. This particular process illustrates well the point that whilst endogenous processes proceed at rates independent of the immediate rate of nitrogen intake, and may usually proceed at remarkably steady rates, yet they do not necessarily proceed at steady rates: if a normal subject becomes a blood donor, some slight increase in endogenous purine metabolism may be expected; if he goes to live at high altitudes a large increase in the rate of this process may be expected to occur rapidly and to be maintained, corresponding with the increase in the erythrocyte count.

Deposit nitrogen:

When attention was given to the course of nitrogen excretion in the period intervening between equilibrium at one level of nitrogen intake and the attainment of equilibrium at a new level of intake difficulties arose in accounting for observed changes in nitrogen excretion in terms of a simple dichotomy into exogenous and endogenous catabolic processes. Fig. 6, taken from a paper by Martin and Robison (1922) illustrates the

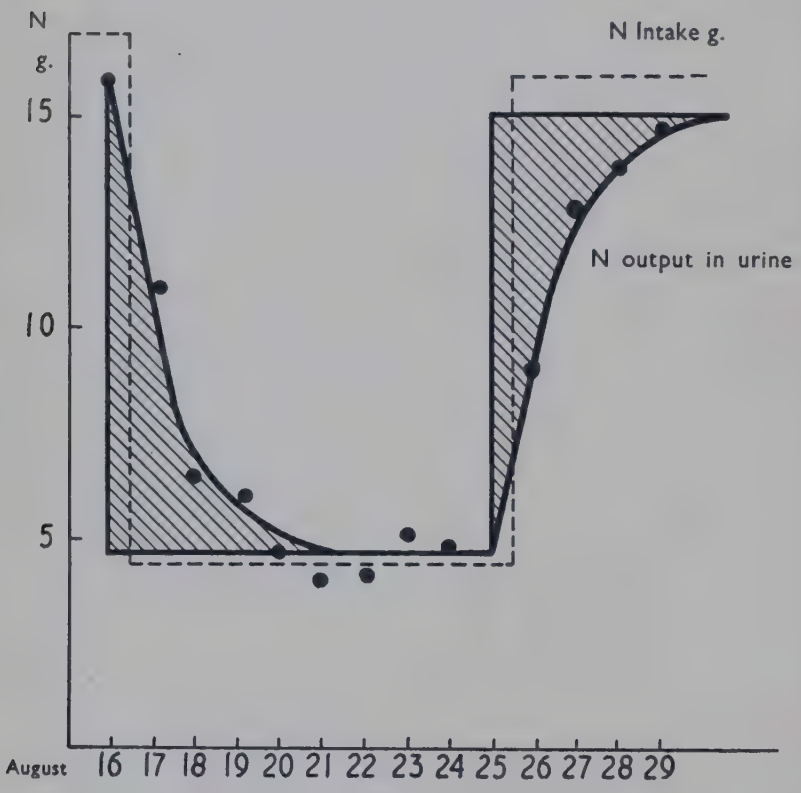

FIG. 6

The effect of change of nitrogen intake on the rate of urinary nitrogen output in a normal human subject (*Martin and Robison*, 1922).

nature of the difficulty. In the transition period between equilibrium on the initial high-protein diet and equilibrium on the low-protein diet to which the subject was transferred nitrogen excretion lagged behind ingestion so that the subject excreted more nitrogen than he ingested. When balance was attained at the low level of intake, the intake was increased to the original level. There was again an excretory lag, and the subject excreted less than he ingested. The excess excretion in the first period was equal to the deficit in excretion in the second period. Similar phenomena were described by Thomas as early as 1910.

This lag process, and the demonstration (see Fig. 7) that the rate of excretion of nitrogen falls off exponentially, seemed to point to the existence of a nitrogen store in the body, the extent of filling of which is a function of the level of protein intake. The exponential

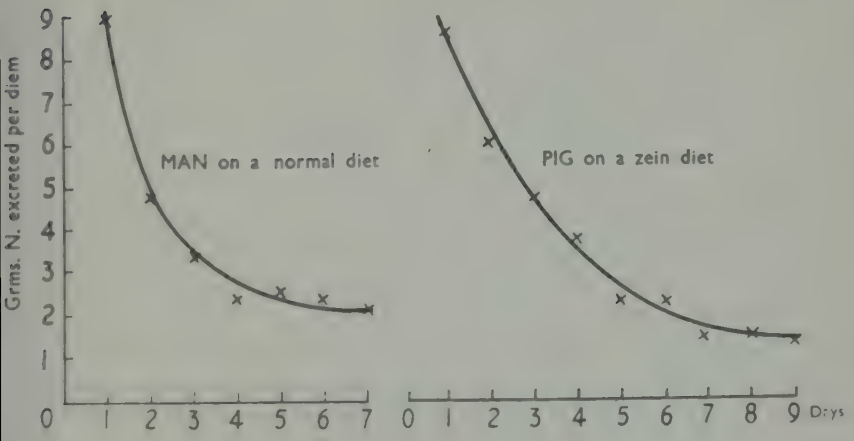

FIG. 7a FIG. 7b

The effect of change from nitrogen-containing to nitrogen-free diet on the rate of excretion of nitrogen in the urine. Fig. 7*a* refers to a man, previously in equilibrium on a normal diet (*Martin and Robison*, 1922). Fig. 7*b* refers to a pig previously on a zein diet (*McCollum and Steenbock*, 1912).

excretion curve suggests that excreted nitrogen is the product of catabolism of a store whose rate of destruction is directly proportional to its mass. This store was originally spoken of as 'deposit protein'. But McCollum and Steenbock (1912) showed that the 'incomplete' protein, zein, which will not support growth, will induce nitrogen storage in the pig. Fig. 7 compares the course of nitrogen excretion in a pig placed on a starch diet after nitrogen storage had been induced by zein with that of a human subject placed on a nearly nitrogen-free diet after being in nitrogen balance on a normal diet. The storage induced by zein is of the same general nature as that induced by a normal diet. Since it was believed that 'incomplete proteins' could not bring about protein synthesis when fed, this finding led to a denial of the existence of deposit protein, though not of the existence of a nitrogen store: the nitrogen store was viewed as essentially non-protein in nature.

'Incomplete' proteins and essential amino-acids:

The proteins designated 'incomplete' were those found to be unable to support growth without supplementation by particular amino-acids which came to be designated 'essential amino-acids'. The implication in the early work was that a dietary protein which failed to support growth was incapable of providing the constituents for the synthesis of tissue protein. We shall see later that the more precise study made possible by modern facilities has led to the demonstration that an amino-acid may be essential for growth but not essential for maintenance of nitrogenous equilibrium in the adult. This does not necessarily mean that the growing animal forms protein and that the adult does not do so, or that what proteins are formed in the adult do not contain certain of the amino-acids. An amino-acid may be a dietary essential even if the animal is capable of synthesizing it, provided that the demand for it exceeds the capacity for synthesis.

Thus, if demand slackens in the adult for certain of the amino-acids, as a result of cessation of growth, it may be possible to meet all demands for a particular 'essential' amino-acid by way of synthesis. In such circumstances the feeding of an 'incomplete' protein could lead to the synthesis of complete proteins by the recipient.

McCollum and Steenbock's (1912) incomplete protein was zein from maize. If this protein is supplemented with lysine and tryptophane it will support growth. Both these amino-acids have been shown to be unnecessary for the maintenance of nitrogenous equilibrium in adults of a number of species. There is therefore no contradiction between the ability of zein to bring about nitrogen storage and the notion that nitrogen is stored as protein.

Continuing nitrogen metabolism:

It appears in retrospect that the puzzle of the nature of the nitrogen store effectively hindered any advance from the Folin endogenous-exogenous concept for many years. The failure to find any non-protein store made difficulties. Borsook and Keighley (1935) were the first to conclude that, despite the apparent difficulties, the store must be protein in nature. They went a great deal further and concluded that by far the largest part of the nitrogen excreted is derived from this store, and that the contribution of truly exogenous metabolism is very small. They believe that the major part of ingested nitrogen goes to build cytoplasmic proteins and that these constitute the 'store'. The cytoplasmic proteins of the various organs are believed to undergo breakdown at characteristic rates, some more slowly and some more rapidly, but each at a rate depending on its cytoplasmic mass. Thus, for this major part of protein metabolism the rate of anabolism is a function of immediate dietary state, but the rate of catabolism, being dependent on cytoplasmic mass, is a function of past dietary history. It is for this

reason that this aspect of protein metabolism is given the name of 'continuing' nitrogen metabolism.

The direct demonstration that the protein content of an organ could be made to rise and fall rapidly by alteration of the protein content of the diet was made by Addis, Poo and Lew (1936a, b, c) and by Murray Luck (1936). Murray Luck also showed that in rat livers in which the total protein content had been increased by 50–60 per cent. by transferring from a 10 per cent. to a 20 per cent. casein diet for seven days, the relative abundance of the different fractions of hepatic protein was not altered, the conclusion being that all major fractions of the liver proteins contributed proportionately to the increase.

Minimum nitrogen requirement:

One of the consequences of the Folin endogenous-exogenous classification was the concept of a minimum nitrogen requirement. On this view of nitrogen metabolism, one ought to be able to subsist on a nitrogen intake sufficient to meet endogenous requirements. This might well include an amount of nitrogen equal to the sum of creatinine nitrogen normally excreted, plus ammonia nitrogen, about half the normally excreted uric acid nitrogen, some fraction of the undetermined nitrogen and some urea nitrogen produced during 'wear and tear' of tissue proteins. Clearly, the urinary nitrogen excretion corresponding to the hypothetical state of just satisfied endogenous needs cannot be calculated theoretically. In order to determine it experimentally one must deplete the body of its nitrogen store, in whatever form it is held. Many investigations were made with the object of determining this minimum level, the general plan being to maintain the animal on a protein-free diet providing sufficient calories to obviate the need to catabolize tissue protein in order to obtain energy. The nitrogen excretion was expected to become steady as

soon as the minimum level was reached. In fact, the nitrogen excretion falls off approximately exponentially for a time, as has already been pointed out, then the rate of decline of excretion alters to such an extent that it has often been doubtful whether any further decline is taking place.

It is of great nutritional and metabolic importance that the significance of the time-course of nitrogen excretion on a nitrogen-free diet should be made clear. There is no doubt that there are at least two processes determining the time-course, and it seems to be most generally supposed that there are three, namely, release of nitrogen from a labile store, release from notably less labile stores, and excretion of end-products of endogenous metabolism.

The work of Addis *et al.* (1936a) showed that the rate of loss of liver protein was far more rapid than that of any other organ: in rats liver lost 40 per cent. of its protein, the alimentary tract, pancreas and spleen 29 per cent., the heart 18 per cent., muscle, skin and skeleton 8 per cent., and the brain 5 per cent. in a seven-day fast. This suggested a possible basis for the composite time-course of nitrogen excretion in protein deprivation. If the protein lost by each organ is a representative sample of the proteins of the organ, i.e. is representative of the cytoplasm of the organ, then the time course of excretion might be expected to be the sum of different exponential functions giving much the sort of appearance as is observed. But it would be difficult to distinguish the time course from that to be expected if the protein lost were of the nature of a 'deposit protein' distributed unequally between different organs, but disappearing at a rate determined by the total amount of it in the body.

The distinction between these possibilities bears on the importance to be attached to the minimum nitrogen excretion. If the extra nitrogen excreted whilst the

nitrogen excretion is falling on a protein-free diet is derived from a deposit protein, then the minimum nitrogen excretion observed may be taken as measuring with reasonable accuracy the nitrogen requirements of normal cells. But if the nitrogen lost is cytoplasmic nitrogen, then the endogenous requirement measured must be that of a diminished cell-mass, and the further question arises whether an animal fed on a nitrogen supply adequate for merely endogenous requirements, and therefore possessing the diminished cell-mass, can be regarded as normally nourished.

The nature of labile proteins:

Murray Luck (1936) was unable to distinguish any change in partition of liver protein between the fractions which could be separated by salting-out procedures, even when the total protein of the liver had been increased to 150 per cent. of normal. Various workers have investigated the change in concentration of readily measurable amino-acids in liver protein following protein starvation, but the results have not been conclusive (see Kosterlitz and Campbell, 1946). Measurements of N : S ratios in liver have also given inconclusive results. For a protein or a mixture of proteins, this ratio gives a reasonably sensitive test of proportion of sulphur-containing amino-acids in the protein. But for an organ or for a crude protein fraction it is far from satisfactory, in view of the large amounts of nitrogen in such cell constituents as nucleic acids, which readily form complexes with proteins. The balance of the evidence would seem to favour the view that the inequalities in rate of disappearance of different protein constituents from the liver is not great enough to warrant the supposition that what disappears is a special deposit protein.

On the other hand the demonstration by Miller (1948) that protein-free feeding diminishes the catalase, alkaline phosphatase, cathepsin and arginase content of the liver

almost in parallel with the loss of protein is positive support for the notion that the labile protein is the working stuff of the cell. Miller (1950) has shown that animals depleted of protein and then refed show a sharp rise in total liver content of the enzymes already mentioned, running parallel with the sharp rise in total liver protein. Many other workers have confirmed and extended Miller's observations (Potter *et al.*, 1947; Seifter *et al.*, 1948; Harkness *et al.*, 1949; Rosenthal *et al.*, 1949; Schultz, 1949). Additional enzymes which have been shown to behave similarly to the total liver protein are succinoxidase, octanoate oxidase, rhodanese, adenosine triphosphatase and D-amino-acid oxidase.

Two points of further interest occur in the papers of Miller quoted above. He has shown for liver arginase that the total hepatic activity is not related to nitrogen intake but to total hepatic protein content by comparing animals on the same intake of nitrogen, in the one case in the form of 25 per cent. casein and in the other in the form of 6 per cent. casein plus 19 per cent. glycine. Liver protein and liver arginase fell in parallel on the glycine diet, although nitrogen intakes were very similar on the two diets. Thus, the level of total hepatic arginase is not to be taken as evidence of arginase formation as an adaptation to the high nitrogen intake and the presumed high requirement for urea synthesis, but is another facet of the lability of the cytoplasmic enzymes.

Miller's second point is of equal importance. We have seen that the lability of the protein of different organs appears to differ. If this be true between organs it would not be surprising if differences in lability were to be found between the individual proteins of a single tissue. Miller's papers show that the xanthine oxidase activity of rat liver falls off far more rapidly than the total protein: a fall in total liver protein of 5 per cent. is accompanied by a fall in xanthine oxidase activity of 80 per cent., on a diet whose only known deficiency is

protein. This fall is promptly reversed by return to high protein diet.

If some enzymes are more labile than the generality of cytoplasmic protein, it is reasonable to suppose that some must prove to be less labile, so that the liver content of them, expressed in terms of the total liver protein, must rise as the liver shrinks. In Table 3 Miller's (1950) data for four enzymes are expressed as relative activities per unit mass of liver protein, the mean relative activity of the control group being taken as 100.

TABLE 3

The effect of change in protein intake on the enzyme activity of rat liver. The Table shows enzyme activity per unit of liver protein (Miller, 1950) expressed as per cent. of the activity found in the control group.

	Control group	*Low protein group*	*Non-protein group*	*Re-fed group*
Catalase	100 ± 15·8 (5)	98 ± 8·6 (6)	74 ± 8·2 (9)	113 ± 18·1 (8)
Alkaline phosphatase	100 ± 3·3 (5)	115 ± 2·8 (5)	148 ± 9·6 (9)	128 ± 7·1 (8)
Cathepsin	100 ± 14·1 (5)	125 ± 6·2 (5)	105 ± 8·8 (9)	95 ± 5·2 (8)
Arginase	100 ± 4·8 (5)	104 ± 7·0 (6)	95 ± 6·0 (9)	92 ± 3·5 (8)

Each estimate of mean activity is followed by the standard error of the mean and by the number of animals in the group.

Looking at these relations, it seems possible that more extended series of observations would show that catalase is more labile than the generality of liver protein, and that alkaline phosphatase is less labile. This suggestion of inequality of lability of the cytoplasmic enzymes is of fundamental importance in nutrition.

It is of great interest that at least two of the ten enzymes mentioned above have been shown to occur in the intracellular granules which can be separated by high-speed centrifugation and possess the staining reactions of mitochondria. These are succinoxidase (Hogeboom, Claude and Hotchkiss, 1946) and octanoate oxidase (Schneider, 1948).

Plasma protein stores:

A different aspect of the relation of the liver to protein metabolism is illustrated by the work of Whipple on plasma protein formation and metabolism. The main features of this work have been summarized by Whipple (1938, 1942), Madden and Whipple (1940) and Whipple and Madden (1944). A technique of *plasmapheresis* was developed to enable blood to be drawn off from an animal and separated into corpuscles and plasma in such conditions that the washed erythrocytes could safely be returned to the animal. The major effect is that of considerable withdrawal of plasma protein from the animal. In a normally well-fed dog the plasma protein concentration returns to normal in a surprisingly short time. Repeated plasmapheresis reduces the speed of recovery, and ultimately the degree of recovery of the plasma protein concentration. Berryman, Bollmann and Mann (1943) showed that no recovery of plasma protein concentration followed plasmapheresis in hepatectomized dogs.

The evidence afforded by plasmapheresis experiments led Whipple to postulate the existence in the body of a pool of dispensable protein, distinct from the indispensable proteins of the cells. In so far as plasma protein formation is concerned, which appears to be dependent on the liver, this would now seem unlikely to be the best interpretation of the findings. It would be of interest to observe the effects of plasmapheresis on liver enzyme activities.

The form of transport of protein in the body:

In carbohydrate metabolism the foodstuff, ingested largely in the form of large condensed molecules, is broken down and absorbed as small units; glucose is transported to liver and peripheral tissues mainly or wholly as small units, but is stored in the condensed form. In fat metabolism it seems that a specialized derivative, a phospholipid, appears necessary for the transport of fat from the liver to other organs, though it does not apparently participate in fat metabolism to any appreciable extent. It is still a moot question whether either of these can serve as a model for protein metabolism. If protein is broken down to amino-acids, absorbed and transported as such, but stored as protein, then it is difficult to see how amino-acids can be preserved from deamination during their entry into the body by way of the portal blood. If amino-acids were transported in a somewhat condensed form, and if the products of protein catabolism differed from the substrates of anabolic processes, much that is now difficult to understand would be made easier of comprehension. The assumption has been made tacitly in much work on protein metabolism that the glucose model is apt, but this is far from being firmly established. It is certainly true that nitrogen balance can be maintained by the intravenous administration of mixtures of purified amino-acids or by acid hydrolysates of good-quality protein supplemented with methionine and tryptophane to replace the amounts destroyed during hydrolysis (see, for instance, Elman, 1940), which suggests that amino-acids can act as a means of transport of dietary protein nitrogen. But it has also been shown repeatedly by Whipple and his colleagues (loc. cit., and Holman, Mahoney and Whipple, 1934) that homologous plasma protein can be utilized by dogs. Nitrogenous equilibrium can be maintained for several weeks, using intravenous plasma protein as the sole source of nitrogen.

Thus there is equally strong evidence for appropriate mixtures of amino-acids and for the plasma proteins as transport forms in protein metabolism. It follows that the physiological form of transport is not established. In particular it ought to be borne in mind that the ability of the organism to utilize efficiently a mixture of amino-acids does not mean that it will or can utilize in the same fashion some single amino-acid fed alone: the normal rapid anabolic processes, in whose reality confidence can now be placed, must involve the condensation of patterns of amino-acids so that the single component of such a pattern fed alone may be expected to be largely denied the opportunity to follow the normal metabolic path.

The access of protein to cells:

The utilization of intravenously administered plasma protein requires acceptance of the view that protein molecules can enter and leave cells reasonably readily. This need not apply to all proteins and all cells: cells with some form of exo-skeleton may succeed in excluding protein, whilst those without such a structure may be expected to incorporate into their limiting layer all species of protein molecule present in the surrounding fluid. Since the limiting layer must be supposed to be in dynamic equilibrium with the cell contents, protein incorporated in the limiting layer may be expected to find its way into the cytoplasm, but such a process would presumably be very slow unless the protein were subject to rapid metabolic transformation within a short time of entry. Thus, provided that a protein could be catabolized, and provided that it could gain entry to the tissue fluid, the question of its entry into cells need not be a barrier to the entertainment of the possibility of protein constituting the normal form of metabolic transport in protein metabolism. It is tacitly accepted that protein can cross limiting layers of cells which

specialize in the production of certain specific proteins, such as those of the saliva, and of mucins of the goblet cells, the insulin of the islets of Langerhans. It has been assumed by some workers that this last protein, insulin, can readily enter those cells whose carbohydrate metabolism it influences. One possible objection to protein as the transport form is therefore not valid. The other objection, namely difficulty of escape from capillaries, is also less serious than might appear. The minute vessels seem to vary widely in their properties from tissue to tissue: the hepatic sinusoids have defects in their walls which permit the free filtration of plasma, the minute vessels of the small intestine appear to permit passage of about half the protein in plasma, those of muscle pass a fluid very poor in protein, but not apparently free from it. Rous, Gilding and Smith (1930) showed that the dye Chicago Blue 6B, which forms a colloidal suspension which can be purified by dialysis, passes quite readily through the capillary wall in muscle. Since particles of this size can pass, it is reasonable to suppose that protein particles can do likewise.

The two tissues with high rates of protein turnover have minute vessels allowing ready access of plasma protein to the cells of the tissue. Muscle, with a lower rate of protein turnover per unit mass, but with equal total importance in protein metabolism, offers in all probability a restricted but real access of plasma protein to the cells of the tissue. These relations do not constitute a demonstration that plasma protein itself is a major transport form, but they indicate that the possibility cannot be dismissed as implausible. When a foreign protein is introduced into an animal it, or some very large fragment possessing its immunological specificity, must gain access to whatever cells are responsible for the production of antibody. This again illustrates the plausibility of the notion of the access of circulating protein to tissue cells.

Tissue protein synthesis:

The broad outline of protein metabolism points to the processes of synthesis of cytoplasmic proteins as central. It is even possible that the rapidity with which labelled amino-acids are incorporated into intestinal mucosal protein and disappear from it again may have to be interpreted as meaning that the process of absorption itself may involve a protein synthesis stage, in which case the products of absorption which enter the body would be expected to be made up of those fragments of food protein which could not be incorporated into intestinal protein plus a specific set of breakdown products of intestinal protein. Until more is known of the physiology of the process of absorption it is not profitable to discuss the details of tissue protein synthesis, but it is of importance to state some of the problems.

If, as we apparently must believe, every enzyme is protein in character, then the body must possess the apparatus for the manufacture of a myriad of specific types of enzyme protein. Whether every type of cell in the body must be capable of synthesis of every type of enzyme which it contains is an open question. Presumably, every type of cell possessing a unique protein must be capable of synthesizing it. Similarly, it is reasonable to suppose that, where the cytoplasmic mass and the total activity of a particular enzyme in a cell possessing labile cytoplasm alter in parallel, then the enzyme is most probably synthesized in the cell. The weight of the indications is in favour of local synthesis of most if not all enzymes.

The apparatus involved in this synthesis—which itself must be supposed to include specific enzymes—must produce from common precursors—the forms of transport of protein—the many types of protein required, at rates which will maintain the character of the cell. There is thus a quantitative as well as a qualitative restriction on the performance of this apparatus. There

can be little doubt that the characteristics of cells are determined largely or entirely by three factors, the nature, intracellular distribution and local concentrations of the cellular enzymes. Any change in total intracellular activity of an enzyme relative to total cytoplasmic mass must mean a change in the second or third of these factors. A cell so changed cannot have the properties of a normal cell. If we consider the dramatic instance of xanthine oxidase of hepatic parenchymal cells, Miller's (1948) finding that its total activity diminishes by 80 per cent. when the total nitrogen of the cell has diminished by only 5 per cent. must be taken to imply that such a cell is not a normal hepatic parenchymal cell. We cannot call it anything other than a hepatic parenchymal cell, and it clearly possesses the capacity to revert to a normal cell of this type when it is adequately nourished. But it has largely lost an item of normal equipment, and the sequence of change undergone by precursors of the substrate of this enzyme must differ profoundly in this cell from the sequence in a normal cell. It is perhaps not surprising that it has long been known that the liver of an animal living on a low-protein diet is hypersensitive to poisons, notably chloroform and other chlorinated hydrocarbons.

The performance required from the synthetic apparatus suggests either that the cell must be equipped with many enzymes with highly specific abilities to condense amino-acids and peptides or that a relatively simple condensing system is governed by secondary agencies of some complexity. The phenomena of immunological reactions suggest very strongly that such 'secondary agencies' exist. The fact that a foreign protein introduced into the circulation can induce in some of the cells of the organism a new type of protein, a specific antibody having specific relations to the foreign protein introduced, implies that the pattern of a protein molecule presented to a cell must in some instances be capable of

xerting an influence on the outcome of protein synthesis n that cell.

All but a very few foreign proteins can act as antigens. The filterable viruses constitute foreign protein-ontaining complexes which can gain entry to host cells nd there excite their own reproduction. In recent years t has come to be believed that the cytoplasm of normal ells may contain complexes of a self-reproducing type of nuch the sort of constitution of filterable viruses. Sonneborn has recently reviewed (1948–49) his work on he kappa factor of *Paramoecium* which is of this nature. Kappa confers on its bearer the property of secretion nto the medium of a substance lethal to normal strains f the species. The self-reproduction of kappa is lependent on the simultaneous presence of the gene in he nucleus appropriate to confer on the cell the power o reproduce kappa, and of one or more kappa particles n the cytoplasm to excite the self-reproduction. These elations suggest that we may find that normal intra-ellular proteins may well act as excitors of self-eproduction, given the presence of appropriate genetic actors. In the vertebrate organism, where many types f cell must be supposed to bear the same genes, the ifferences between cells must be supposed to depend on ther than genetic factors, on such matters as the in-qualities in their shares of the cytoplasm of the original vum, and on the secondary consequences of these nequalities, the differences in their environments.

nternal environment and protein metabolism:

All four major factors of the internal environment of a issue may be expected to modify the pattern of its rotein metabolism. We may consider the factors in the rder of pH, oxygen tension, osmotic properties and utrient supply.

Cells in general are markedly sensitive to changes in ie pH of the fluid in which they lie. In view of the

strong buffering capacity of the cytoplasm it seems unlikely that there can be any shift in intracellular pH comparable with the shifts in extracellular pH which can occur in physiological conditions, but it is difficult to believe that no shift at all occurs in intracellular pH. Even a small change is likely to have an effect on the metabolic pattern of a cell. Since the pH-optima of enzymes appear to scatter around the physiological pH range, small shifts of pH will increase the activity of some enzymes, decrease that of others and leave that of the rest unchanged. No very great shift of pH ought therefore to be necessary to perturb markedly any metabolic apparatus dependent on the successive functioning of series of enzymes.

The extracellular pH may be expected to have a second kind of effect on cell function: it is to be expected that it will modify the degree of ionization of constituents of the boundary layer of the cell, which will cause changes in the pattern and the properties of this layer as a whole, a possible consequence being alteration of the permeability of the layer, which, by modifying exchange of metabolites between the cell and the environment could modify the pattern of metabolism.

The bearing of oxygen tension on metabolism is also complex. In addition to its influence on the rate of production of metabolites which result from oxidative processes, it will also determine the extent to which sulphydryl groups of proteins will be free. In approximate terms, we may regard a protein as consisting of one or more peptide chains, with secondary linkages between amino-acid side-chains of the same or of different peptide chains. The chemical properties of the protein will depend on the nature and arrangement of the residual free amino-acid side-chains presenting to the exterior of the molecule. A particularly important secondary linkage is between two cysteine molecules by way of their -SH groups. An oxidative reaction can occur,

yielding an -SS- link. A very large number of enzymes is now known which are capable of exhibiting their catalytic activity in one of the two forms -SH or -SS- but not in the other. The equilibrium between the two forms is known to be dependent on oxygen tension for some of the known intracellular proteolytic enzymes, so that we may expect changes in oxygen tension to have effects similar to those to be expected following pH changes, additionally to the specific effects of oxygen lack on metabolism.

Osmotic effects need not receive much attention. Where a cell undergoes hydration or dehydration all its contained enzymes and many of their substrates undergo corresponding dilution or concentration. We may neglect changes in enzyme concentration: the volume of the system changes in complementary fashion. Change in substrate concentration will have a very different effect: depending on the nature of the kinetics of the reaction and the degree of saturation of the enzyme there may be in particular instances no change in reaction rate, or increase or decrease in rate in response to a dilution of the substrate. Here again, there is reason to suppose that the metabolic pattern will be disorganized.

In the special instance of protein metabolism, in which we envisage synthesis of the set of cytoplasmic proteins as the first step in the transformation of protein nutriment, we may expect competition between processes leading to different end-products to result in an end-product pattern depending on the rate of supply of nutriment. Thus all the major environmental factors may be thought of as tending to alter the pattern of protein synthesis in the same general way, though each will have its own particular mode of expression.

Physiological influences on protein metabolism:

These considerations should make it clear that protein metabolism is liable to be influenced by factors other

than the rate of ingestion of protein. The processes of growth and differentiation of tissues are witness to periods of constantly changing cellular protein metabolism, but equally as striking phenomena occur in the fully-grown animal. The stability of size of organs in the adult might seem in general to be satisfactorily accounted for if one were to assume that differentiation proceeded to a point at which power of cell-division or of increase in cell mass, or both, were finally lost. But for some organs this cannot be an explanation. We have seen that the liver is able to change its cytoplasmic mass over a range the extremes of which bear a ratio to one another of the order of three to one. It is also a well-known phenomenon that if a considerable part of the liver be removed surgically from an adult rat, the residue will grow by increase in cell number until it reaches approximately the normal hepatic mass. This increase in cell number occurs as a result of an outburst of mitosis in all parts of the residual liver tissue: growth is not confined to or even especially obvious in the zone of injury. Here then we have a tissue which retains a capacity for cell-division and normally exhibits a capacity for increase in cell mass. But normally the cell number is stable and the cell mass fluctuant only within narrow limits. Nevertheless, removal of a part of the organ which is too small to impose any obvious physiological hardship on the animal results in systematic stimulation of cell-division in the rest of the organ.

A somewhat similar phenomenon was demonstrated by Sanders and Florey (1940) who devised ingenious and elaborate methods for the location and surgical removal of lymphoid tissue from rats, and found that when all the lymphoid tissue which they could identify had been removed the animals still had normal lymphocyte counts and were in no way incommoded. The explanation was found in the discovery deep in the liver of a large mass of lymphoid tissue which is not present in normal animals.

Here we are concerned with a tissue normally exhibiting cell-division, resulting in lymphocyte production, this process occurring, if lymphocyte count may be taken as a rough indication, at an approximately steady rate. The tissue, however, is scattered through the organism in a large number of separate fragments. The residual fragment reacts to the removal of the rest in much the way that the hepatic residue reacts to the removal of the rest of the liver. In these instances it would seem probable that the elements of the tissue are competing for some nutrient of which the supply is limited; whether this is so, and whether the substance in question is properly designated a nutrient must remain uncertain: what is clear is that the growth activity and consequently the protein metabolism of one part of an organ or one fragment of a scattered tissue is under the influence of the rest of the organ or tissue.

Another sort of physiological influence on protein metabolism is illustrated by the changes in muscle mass which are brought about by exercise or by refraining from exercise. There is no doubt that immobilization can bring about reduction of muscle mass to vanishing point or that systematic exercise can convert a normal man into a typical 'strong man'. The effect is local, not systemic, since a painful injury which, for instance, renders use of one lower limb painful but does not immobilize the subject, will result in a well-marked relative atrophy of the painful limb. It is also a well-known phenomenon that particular muscles which are systematically used show marked hypertrophy.

No large change in mass of a muscle can take place without corresponding change in protein content: protein constitutes by far the largest part of the solids of the tissue, and the hypertrophy or atrophy of a muscle must be regarded as a primary or secondary alteration in its protein metabolism. In searching for a physiological basis for this phenomenon it is of interest to consider the

factors regulating the distribution of blood within muscles. It is known that muscular activity is accompanied by an enormous increase in the number of patent capillaries (Krogh, 1922) and it has been pointed out that this means a diminution in the mean distance between tissue cells and blood capillary, with a corresponding increase in the rate of exchange of solutes between blood and cells. Since the tissue does not apparently suffer appreciable anoxia nor become appreciably more acid, it is supposed that capillary dilatation is probably governed by oxygen requirement or CO_2 production. These two are closely related to one another normally, so that regulation in conformity with one of these requirements will approximately satisfy the other. But only one metabolic feature of the muscle can govern the distribution of blood within it: the circulation through the muscle must be regarded as being unregulated or disregulated with respect to every other metabolic requirement.

The particular application of this to the present problem is that since exercise has no necessary relation to the concentration of protein, peptide or amino-acid in the plasma, there will be no relation between the concentration of protein nutrients in the blood and the occurrence of phases of more rapid exchange between blood and muscle cells. In these phases the protein nutrition of the cells may be supposed to be more generous than usual, and hypertrophy may be the natural passive consequence of this. Correspondingly atrophy might be accounted for by diminution in normal quiet tonic activity. This involves the acceptance of the notion that a distribution of blood within a muscle might well be adequate to supply oxygen for resting metabolism whilst being inadequate to supply the protein nutrients needed to maintain the normal form of the cell.

These examples serve to illustrate ways in which interaction between cells and interaction between

physiological activities of a single type of cell can be expected to modify the course of protein metabolism. The animal is not a mobile test-tube, and care must be taken not to think of problems of intermediary metabolism solely in terms of chemical reaction schemes.

Conclusion:

Folin's concept of the division of nitrogen metabolism was very soon questioned, since it did not account for the large lag of change of nitrogen excretion rate behind change of nitrogen ingestion rate. The notion of a form of deposit protein was suggested, but appeared inconsistent with the ability of animals to store nitrogen fed in the form of incomplete proteins not capable of supporting growth.

The independent work of Borsook and Keighley and newer knowledge of the nutritional significance of the amino-acids have established the reality of deposit protein and removed the former difficulties in the way of accepting the notion. More recent work has established that liver and intestinal mucosal protein are the most labile in the body, with muscle next. Many views have been expressed concerning the nature of deposit protein, but the demonstration by Miller and by others that many liver enzymes appear and disappear at rates of the same order as that of loss or gain of liver protein suggests very strongly that 'deposit protein' is not a specialized storage product but the cytoplasm itself.

The weight of evidence is in favour of the view that most incoming nitrogen is condensed to form tissue proteins and that catabolic processes are secondary to this anabolic one. Some nitrogen in unutilized forms (e.g. purines, creatinine) may pass immediately out of the body. A little may be used directly for endogenous processes. Erythropoiesis has been cited as an instance of a physiological activity involving endogenous nitrogenous metabolic processes.

The processes of tissue protein synthesis are clearly of prime importance in protein metabolism. Some probable general properties of these processes are that they involve competition between end-products for the common precursors and that there are almost certainly secondary influences (other than the supply of precursors of protein) which determine the direction of the synthetic processes.

Finally, it has to be remembered that protein metabolism is a physiological problem, and that such local factors as the internal environment of the tissue and local variations in distribution of blood within an organ can in theory have profound effects on the direction and extent of protein synthesis. Protein metabolism cannot be described adequately in terms of chemical reaction schemes.

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CHAPTER III

DEAMINATION, TRANSAMINATION AND UREA SYNTHESIS

In some invertebrates nitrogen is excreted mainly as ammonia, in insects, reptilia and birds it is excreted largely as ammonium urate, in most fish, in amphibia and in mammals it is excreted largely as urea. Needham (1931) has shown that in the chick embryo there are successive phases of ammonia, urea and urate excretion, i.e. there is chemical as well as morphological support for the 'recapitulation' hypothesis of embryonic development. From the present point of view it is more important that there is a suggestion of some chemical community between the processes leading to the major nitrogenous excretory products.

Since animals are capable of ready transformation of administered ammonia to urea or urate, depending on species, the assumption is often made that ammonia is a normal intermediate in the formation of these end-products, and that the deamination of amino-acids is an essential stage in protein metabolism. The assumption is supported in a fashion by two lines of evidence. The first is that it is believed that certain amino-acids can be converted quantitatively or preponderantly to ketone bodies or to sugar in the body. suggesting that, as a first step, they lose their nitrogen but otherwise retain their molecular integrity. The second line of evidence is that enzymes capable of catalysing the deamination of amino-acids have been demonstrated in liver and kidney extracts. The value of the first of these lines of evidence will have to be considered in the next chapter. For the present it may be said that it is not great.

The metabolic significance of specific enzymes:

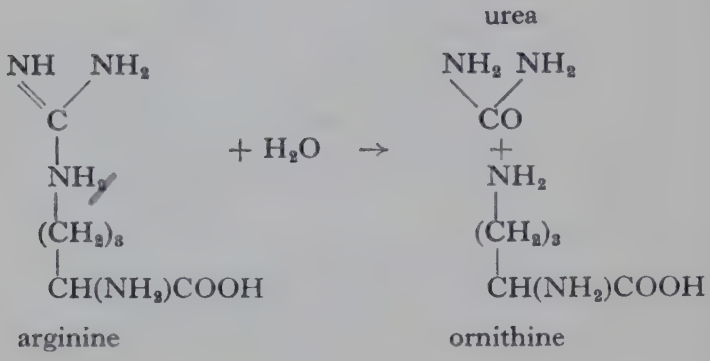
In general, the demonstration that a tissue preparation or extract possesses the ability to catalyse a particular reaction contributes only this much to the physiological probability of the reaction: it demonstrates that it *could* occur. One can demonstrate the existence of an enzyme only by the deliberate introduction into a tissue preparation of an arbitrarily chosen substrate in conditions in which some appropriate measure of chemical change can reasonably be accounted for only by the catalytic transformation of the substrate introduced. Before one can claim that the biochemical potentiality of the tissue which has been so demonstrated represents a normally prosecuted physiological activity one must show that the substrate in question is normally available in sufficient concentration to ensure that the reaction proceed at a physiological rate. It would also be desirable, but is out of the question in most instances, to establish two negative propositions (i) that the tissue is not likely to contain in physiological circumstances substances capable of competing effectively with the chosen substrate for the enzyme, and (ii) that the suggested direction of reaction is not likely to be reversed in physiological conditions by the influence of secondary reactions.

Physiological control of direction of reaction:

Equilibria rarely occur in the body. Any chain of metabolic processes starts with the introduction of new molecules into the body and ends with their removal as excretory products by way of one or more of the lungs, kidney, intestine or skin. The chain of potential equilibria between the successive stages in the transformation from foodstuff to end-product is continually being displaced by new formation of substrate for each transformation and by continuous removal of each transformation-product. An example may make clear

the possible significance of this physiological relation between enzyme-catalysed reactions. The enzyme arginase is widely distributed in the vertebrates, but the arginase activities of most tissues are very low compared with that of mammalian liver. In birds the extra-hepatic tissues have the same trivial arginase activities as the corresponding mammalian tissues, but there is also little or no arginase in the liver. Arginase catalyses the reaction:

$$\text{Arginine} + \text{Water} \rightleftharpoons \text{Urea} + \text{Ornithine}$$



and the equilibrium attained when this catalysed reaction proceeds in a closed system corresponds to almost complete hydrolysis of arginine.

There are two chemical-physiological differences between birds and mammals which might conceivably depend on the different arginase endowments of the phyla. Mammals excrete the major part of their waste nitrogen as urea which they obviously synthesize within their bodies. It is doubtful if birds form any urea at all. There is no difficulty in seeing how this difference might be related to the different arginase contents of the livers. The other difference between

ne phyla is that whereas Scull and Rose (1930) showed nat rats can synthesize large amounts of arginine, nicks (Klose, Stokstad and Almquist, 1938) appear be entirely dependent on an external supply of rginine, i.e. they have very little or no power to syn-nesize it.

If we are prepared to assume (*a*) that ornithine and rea can be produced from other sources in the mammal, nd (*b*) that any arginine formed by catalytic condensa-on of these two compounds in the presence of arginase ill be promptly removed by a secondary reaction, such the reactions which undoubtedly occur which result the appearance of administered arginine in the liver roteins, then we can say that the difference between rds and mammals in their capacity to synthesize rginine could be accounted for by their different hepatic rginase contents.

Thus the demonstration in tissues of a particular nzyme can be made to 'account for' either of two fferent physiological processes, the two requiring pposite directions of catalysed change. The demon-ration of an enzyme in the isolation of a biochemical xperiment does no more than provide us with one of e necessary steps in the demonstration of the chemical-hysiological process of transformation, and we shall see at this caution is well justified when we consider the nzymes which have been demonstrated in the study of e deamination of amino-acids.

-amino-acid oxidase:

rebs (1933) was the first to demonstrate that mam-alian tissues, particularly liver and kidney, possess the wer to oxidize amino-acids to the corresponding to-acids and ammonia. This work produced the zzling answer that these tissues were far more effect-e in the deamination of the 'non-naturally occurring'

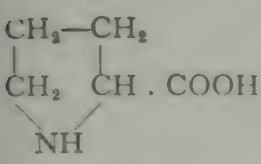
D-amino-acids than of the naturally occurring L-amino-acids.

'Non-naturally occurring' is no longer an exact description of D-amino-acids, but it seems likely that it is true for the mammalia in which the D-amino-acid oxidase occurs. The only substantiated findings of D-amino-acids in nature have been in bacterial peptides.

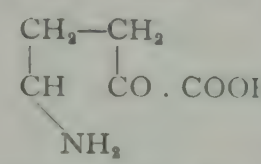
The discovery of the D-amino-acid oxidase was due to the accident that it is relatively difficult to obtain pure optically active amino-acids, so that first attempts to study deaminating enzymes were made with synthetic amino-acids which are mixtures of D- and L-forms. We must suppose either that the D-amino-acid oxidase is an accidental by-product of tissue activity having no physiological activity, or that it is an enzyme capable of catalysing some other reaction in addition to catalysing the oxidative deamination of a substrate which it is almost certain never to encounter physiologically. We certainly cannot assume that because certain tissues have been shown to contain an enzyme capable of catalysing the deamination of D-amino-acids therefore deamination of D-amino-acids is a normal metabolic process.

L-amino-acid oxidase:

More recently Blanchard, Green, Nocito and Ratner (1944: 1945) have prepared from rat liver and kidney an enzyme which will deaminate certain L-amino-acids. This enzyme is a flavoprotein, whereas the D-amino-acid oxidase is flavin-free but requires flavin-adenine dinucleotide as a co-enzyme. The L-amino-acid oxidase oxidizes all the L-amino-acids known to occur in proteins except glycine, serine and threonine, and the basic amino-acids lysine and arginine. It also oxidizes the imino-acid proline, without deaminating it, yielding an δ-amino-acid, α-keto-δ-amino-valeric acid, which is not



proline



α-keto-δ-amino-valeric acid

rther oxidized. The capacity of the enzyme to oxidize -amino-acids is low, even in rat tissues, which are latively rich in it, and it is doubtful if it can account r the physiological rate of protein catabolism. In ddition, an unexpected property of the enzyme has een uncovered which complicates the interpretation of s physiological role. Blanchard *et al.* (1945: 1946) ccidentally discovered that purified preparations talysed the oxidation of α-hydroxy-acids more rapidly han that of α-amino-acids. Since the ratio of activity wards the classes of substrate remained constant uring purification, it seems very likely that the two ctivities are properties of the same enzyme molecule. Lactic acid is oxidized much more rapidly by the nzyme than the most rapidly oxidized amino-acid. ince lactic acid is likely to occur in tissues in a con- ntration as high as that of the most plentiful of the mino-acids, and will presumably compete with them, hilst they in turn compete with one another for the ailable enzyme, there are grounds for supposing that physiological conditions the enzyme will have little ance to deaminate amino-acids.

Here again, as with the D-amino-acid oxidase, it is oubtful if the name given to the enzyme is biologically tisfactory, even though it is historically accurate. It becoming a commonplace matter nowadays to find at enzymes have a wider range of catalytic activity an had been supposed. Just as the L-amino-acid idase oxidizes two groups of chemically similar but ochemically distinct compounds, so it has been shown

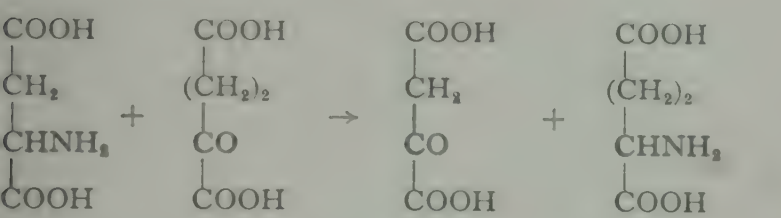
that endopeptidases will not only hydrolyse specific peptides, but will also hydrolyse related esters, the esters being hydrolysed some 20 to 30 times as rapidly as the corresponding peptides (Schwert, Neurath, Kaufmann and Snoke, 1948; Kaufmann, Schwert and Neurath, 1948). Enzyme specificities seem likely to prove to be based much more closely on the chemical considerations of molecular shape than on biochemical considerations of similarity of metabolic role. The accident that an enzyme is first demonstrated and defined with the aid of a substrate of one biochemical class does not at all necessarily mean that the biological importance of the enzyme is in any way related to that biochemical class of substance; the indophenol oxidase and the D-amino-acid oxidase ought to stand as reminders of this.

Transamination:

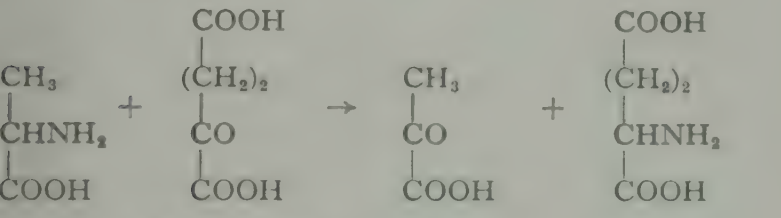
The evidence for the occurrence of deamination of amino-acids by reactions in which the individual acids are oxidized to keto-acids and ammonia is largely indirect. All that we know quite definitely is that the nitrogen of protein, which is preponderantly α-amino-nitrogen, is finally detached in a form which is preponderantly urea. The detachment presumably occurs during the catabolism of molecules which are presumably smaller than protein molecules, but this process could conceivably occur in some way other than oxidative deamination.

Braunstein and Kritzman (1937) provided evidence for such a process when they described 'transamination' reactions in tissue extracts. In such a reaction an amino-acid $R_1CHNH_2 . COOH$ reacts with a keto-acid $R_2CO . COOH$ to yield the keto-acid $R_1CO . COOH$ and the amino-acid $R_2CHNH_2 . COOH$. Provided that several amino-acids are capable of reacting with a single

keto-acid for which a specific active deaminase or other amino-transfer catalyst exists, such transamination reactions could provide plausible alternatives to specific deaminations.



spartic acid | α-ketoglutaric acid | oxaloacetic acid | glutamic acid



lanine | α-ketoglutaric acid | pyruvic acid | glutamic acid

Earlier attempts to define the scope of transamination reactions were not very promising. Green, Leloir and Nocito (1945) purified two different enzymes, which catalyse respectively the reactions:

spartic acid + α-ketoglutaric acid ⇋ oxaloacetic acid + glutamic acid

lanine + α-ketoglutaric acid ⇋ pyruvic acid + glutamic acid

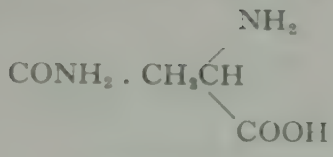
ohen and Hekhuis (1941), after an extensive survey f the transaminase activities of different tissues, con-uded that the first of the above reactions was the only

one which was likely on quantitative grounds to be of any physiological importance. More recently Cammarata and Cohen (1950) have shown that aqueous extracts of minced rabbit heart, kidney and liver can form glutamate from α-ketoglutaric acid and a number of amino-acids. In heart the branched-chain acids, leucine, isoleucine and valine, undergo transamination at rates exceeding 40 per cent. of the rate for aspartic acid; in liver only tyrosine and arginine undergo transamination at rates of 40 per cent. or more of that of aspartic acid; in kidney the nearest approaches to aspartic acid are leucine (30 per cent.) and arginine (35 per cent.). The rates of aspartic acid transamination are similar in all three tissues, and those amino-acids other than the ones mentioned above for which transamination was demonstrated were transformed very slowly. No firm evidence of transamination was obtained for serine, histidine, glycine, threonine, lysine or cystine.

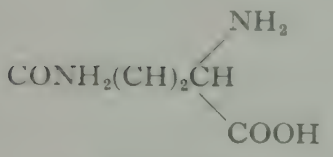
In these experiments the aqueous tissue extract was freeze-dried, and the experiments carried out with a solution of the solid so obtained. The concentration of extract constituents so obtained was of the order of ten times that to be expected in experiments with fresh tissues. In addition a large amount of pyridoxal phosphate, a coenzyme of Green's transaminases, was added in all experiments. It is therefore difficult to make any judgment of the possible physiological bearing of the transaminations demonstrated, and Cohen and Hekhuis's (1941) earlier conclusion that only the aspartate reaction is likely to be of any physiological interest is still probably not far wrong.

Glutamine:

One other possibility, which still remains largely unexplored, is that the amino-N of the amino-acids may be transferred to the second carboxyl of the dicarboxylic



asparagine



glutamine

acids to form the acid amides glutamine and asparagine. These compounds are plentiful in nature, and it has long been known that they are formed and decomposed with considerable readiness in plants. Krebs (1935) found that tissue extracts are capable of condensing ammonia, or the amino-groups of certain amino-acids, with glutamic acid to form glutamine. It is also of interest that Hamilton (1945) has shown recently that a very high proportion of the soluble amino-N of blood plasma, both in the dog and in man, is present as glutamine. We must not attach too much weight to this demonstration: a high concentration of a blood constituent may as readily signify difficulty in utilizing it as ease in making it. The evidence for the physiological role of glutamine is far from complete, but it may prove that a solution to some of the difficulties of accounting for 'deamination' lies in this direction.

Thus, although we are certain, physiologically speaking, that the greater part of all α-amino-N introduced into the body in the form of proteins ultimately leaves the organism in the form of nitrogenous end-products which can be synthesized by the body from administered ammonia, yet it is not at all clear that amino-acids are in general simply deaminated to yield keto-acids and ammonia. One other possibility, that of transfer of

α-amino-groups from L-amino-acids to keto-acids, the transamination reaction, has been shown to be of restricted scope. The possibility that these processes and that of glutamine formation might between them account for the transfer of amino-groups from all the different naturally occurring amino-acids requires to be examined before moving to other topics. The L-amino-acid oxidase oxidizes leucine, methionine, and isoleucine relatively rapidly, phenylalanine and tryptophane about half as rapidly and valine, tyrosine, alanine and cystine about half as rapidly again. The only further amino-acids deaminated at any appreciable rate by the trans-amination process are aspartic acid and arginine, and there is no indication that glutamine can be formed by transfer of amino-groups directly from amino-acids. Speck (1949) has shown that glutamic acid will condense with hydrazine, hydroxylamine and simple amines in the same way as with ammonia, but this is apparently not a specialized aspect of synthesis of γ-peptides since the enzyme system responsible will not yield condensation-products of glutamic acid with any of the α-amino-acids.

Apparently, then, nine amino-acids can be directly deaminated at an appreciable rate, provided that there is no competition for the enzyme by hydroxy-acids, and two others can possibly transfer amino-N to glutamic acid, which does not figure in the list of readily deaminated amino-acids. The glycine oxidase of Ratner, Nocito and Green (1944) may account for the catabolism of one more amino-acid, but the deamination of more than half the amino-acids known to occur in animal tissues is still not accounted for, and the fate of the remainder depends on the ability of the organism to deaminate glutamic acid. It has been shown by von Euler, Adler, Gunther and Das (1938) that rat tissues contain a 'glutamic acid dehydrogenase' capable of catalysing the oxidative deamination of glutamic acid.

The enzyme is present in many tissues, but the activity of liver and kidney extracts is much higher than that of other tissues. Although these authors describe the enzyme as a glutamic acid dehydrogenase, they point out that the equilibrium point is so far in favour of glutamic acid that the enzyme should probably be considered as concerned in the synthesis of glutamic acid from α-ketoglutaric acid and ammonia, rather than in deamination of glutamic acid. It has already been pointed out that position of equilibrium is a poor guide to physiological direction of transformation. It would be of interest to know what are the physiological concentrations of glutamic acid and of α-keto-glutaric acid in liver and kidney. It is now generally accepted that α-ketoglutarate is constantly being formed in carbohydrate metabolism, as a component of the tricarboxylic acid cycle. This provision of the product of deamination from extra-protein sources has to be borne in mind as a factor biasing the direction of the catalytic activity of the 'glutamic acid dehydrogenase' towards amination rather than deamination.

As the direct attack on the problem of the mode of splitting off of α-amino-N from protein does not give a clear answer, indirect attack has to be attempted. An approach can be made by studying the mode of formation of the excretory end-products. We therefore turn to the mechanism of urea synthesis.

Urea synthesis:

Although it was clear that in the mammal the greatest part of the ingested nitrogen was converted into urea before it was excreted, the state of knowledge in 1932 was still as it had been when McCance (1930) showed that the sole unequivocally demonstrated urea-yielding process in any animal tissue preparation was the hydrolysis of arginine by liver. Since the source of the urea nitrogen in this instance was the terminal guanidino-

group of the arginine, there was no known path for the conversion of α-amino-N to urea.

It was therefore a matter of very great interest when Krebs and Henseleit demonstrated in 1932 that ammonia could be converted to urea by slice preparations of surviving liver cells, and that this conversion was catalysed by ornithine. Krebs also showed that the amount of urea produced was increased when citrulline was added, though he did not formally demonstrate that this effect was a catalytic one. In the one instance quoted in the paper 11·4 μM of citrulline gave rise to 4·4 μM of urea. Gornall and Hunter (1943) examined the relation between rate of urea production and concentration of added citrulline or ornithine. The data of Table I of their paper show that when much lower concentrations of citrulline are used than the 200 mg./100 ml. used by Krebs and Henseleit, the citrulline effect is still not necessarily catalytic. Although the experiments quoted in Table I of Gornall and Hunter showed ornithine and citrulline effects which were greater than average, the highest yield of urea relative to citrulline was of 4·7 μM extra urea as the result of adding 4·6 μM of citrulline to give an initial concentration of 18 mg./100 ml. The citrulline effect seems to have been accepted as catalytic without establishment of its nature.

This is of central importance, since Krebs put forward a scheme to account for urea synthesis which gives citrulline a chemical catalytic role of equal importance to that of ornithine. The scheme of reactions proposed was:

$$\text{Ornithine} + CO_2 + NH_3 \longrightarrow \text{Citrulline} + H_2O$$
$$\text{Citrulline} + NH_3 \longrightarrow \text{Arginine} + H_2O$$
$$\text{Arginine} + H_2O \longrightarrow \text{Urea} + \text{Ornithine}$$

Provided that it was correct to assume that amino-acids are normally deaminated to give free ammonia, this scheme could be made to account very readily for the

peculiar position described by McCance (loc. cit.), namely that it was impossible to demonstrate urea production in liver tissue preparations from any source of α-amino-N, the only demonstrable urea-producing reaction being hydrolysis of the arginine guanidino-group. One had only to suppose that ornithine was usually washed out of liver tissue or destroyed in the course of preparation of the tissue for experiment to explain the earlier failures.

Conditions for demonstrating the ornithine cycle:

There are still some difficulties in accepting the cycle of events proposed by Krebs as a basis for physiological synthesis of urea. The most fundamental of these difficulties arise out of the experimental conditions necessary to give the ornithine effect. As one might expect, Krebs found that liver cells had to be supplied with energy in order that they might prosecute a synthetic reaction. Table 4 which is derived from Krebs and Henseleit's (1932) data, shows the effect of different possible sources of energy on the rate of urea production in the presence of ornithine and ammonia.

TABLE 4

The effect of 'nutrients' on the rate of urea formation from added ammonia in rat liver slices

Q_{urea} *in presence of*	*Nature of 'nutrient' added*				
	None	*Lactate*	*Pyruvate*	*Glucose*	*Fructose*
No ornithine	2·1(3)	2·5(3)	2·5(3)	3·0(3)	3·1(3)
Ornithine	3·8(2)	12·5(2)	9·0(1)	5·6(1)	9·2(1)

The figure in parentheses is the number of observations in each set.

Two points are to be noted particularly in this Table. The first point is that a marked catalytic effect of ornithine occurs only in the presence of very high concentrations of lactate or pyruvate. Normal lactate concentrations in body fluids are likely to be of the order of 10 mg./100 ml. and normal pyruvate concentrations are much lower. It is unlikely that lactate concentrations will rise as high as 100 mg./100 ml. in the course of an average human daily life. Thus, even if the unnatural isomer in the racemic lactate used by Krebs can be neglected, the conditions for Krebs's synthesis are highly unphysiological.

The second point is that in the experiments referred to in Table 4, Krebs used 12 mg. of ammonia per 100 ml. of medium, and this concentration or 18 mg./100 ml. was used in most of the experiments. Naturally, it was necessary to provide enough ammonia to permit of the formation of a convincing amount of urea. Such an amount of ammonia necessitated concentrations of the order of 10–20 mg./100 ml. in the small volumes used. But the normal concentration of ammonia in blood, and therefore presumably in plasma and tissue fluids, is a small fraction of a milligram per 100 ml., and this is important, since Krebs's own data on the relation between ammonia concentration and rate of urea synthesis show the great interdependence of the two. The relation is shown in Fig. 8. The curve fitted to the data is of a type consistent with the enzymatic nature of the process. Its precise form is not of importance, since if we take it that there is likely to be a progressive smooth fall in urea formation as the initial ammonia concentration falls from the lowest level used by Krebs to zero, we shall get much the same estimates for probable rate of urea synthesis at low ammonia concentrations whatever the form of curve chosen.

If, for instance, we adopt the cautious position of Peters and Van Slyke (1931, Chap. VI), and assume that

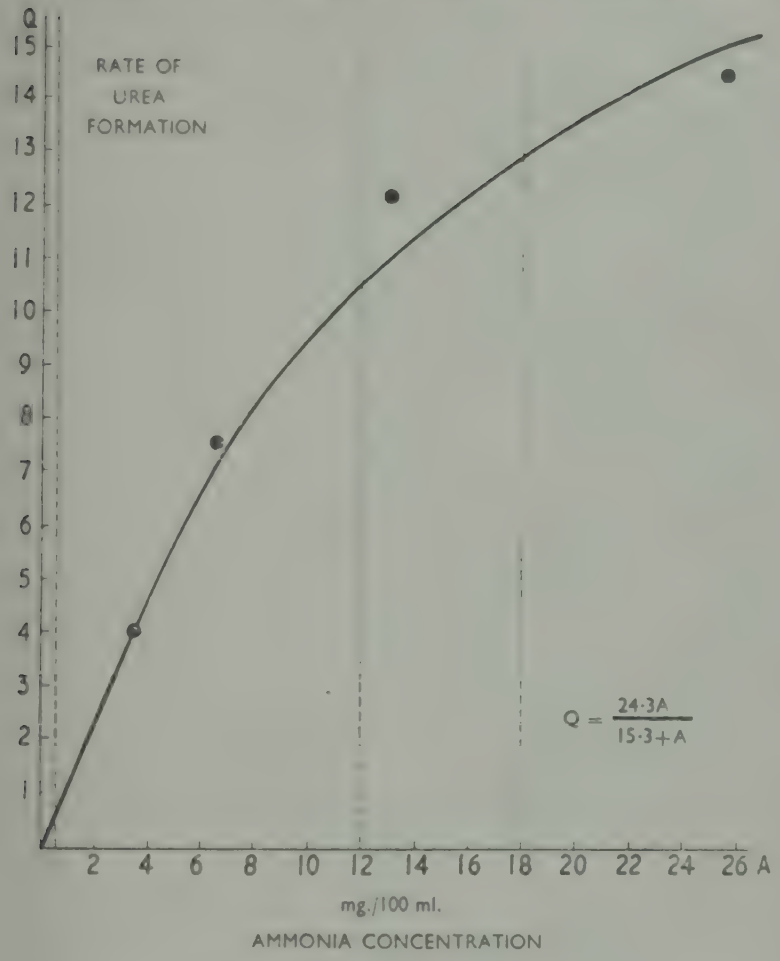


FIG. 8

The relation between the rate of urea formation in liver slices n the presence of lactate, ornithine and ammonia and the con- entration of ammonia. The curve fitted is of a Michaelis- Menten type. (*Based on the data of Krebs and Henseleit* (1932).)

he ammonia concentration in body fluids may be as igh as 0·3 mg./100 ml. in physiological circumstances, he corresponding value of Q_{urea} may be estimated to e in the region of 0·4. This means 0·4mm³ of CO_2

formed by the hydrolysis of the urea produced by 1 mg dry weight of liver in one hour. The experimental conditions are such that hepatic cells may well be functioning with less than physiological vigour, but on the other hand conditions have been weighted in their favour by providing an unphysiologically high lactate concentration and also probably a more favourable ornithine concentration than might occur in the body.

So far as the ability of the rat to synthesize urea *in vivo* is concerned we may take it (see for instance Campbell and Kosterlitz, 1948) that a 100 g. rat can catabolize at least 2 g. protein a day, of which at least 80 per cent. will be excreted as urea. Taking the liver as 4 per cent. of the body weight and the dry weight of the liver as 25 per cent. of the wet weight, this gives an average Q_{urea} of over 8. Since nitrogen excretion does not proceed uniformly throughout the day, but shows peaks succeeding feeding periods, the maximum Q_{urea} will be distinctly higher than this. There is no good way of estimating how much higher it may be, nor is there profit in doing so: all that requires to be established is that there is a large disproportion between the Q_{urea} necessary to meet physiological requirements and that likely to be exhibited by the ornithine cycle in liver slices when it is presented with physiological concentrations of participating and accessory substances. This large disproportion does exist, and requires to be accounted for before the cycle can be accepted as a major source of urea in physiological conditions.

Effects of excess ornithine:

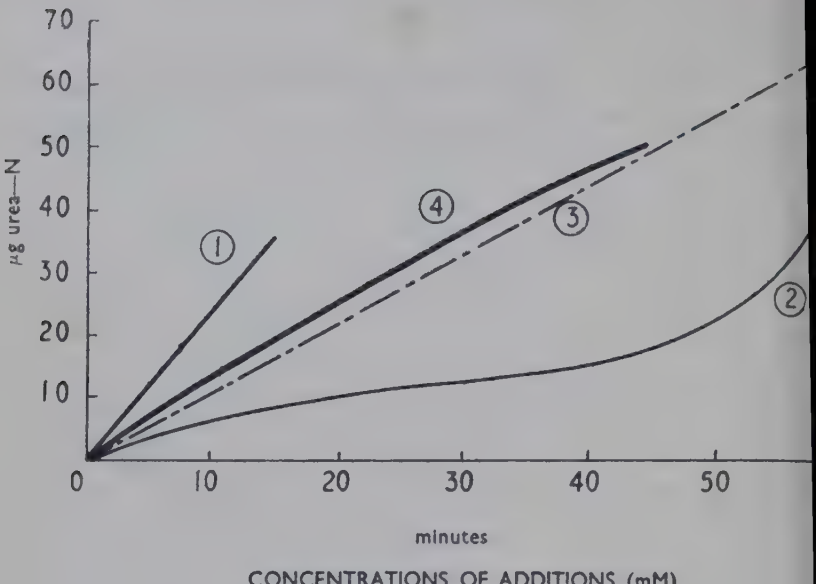
There are later observations on the Krebs scheme which reveal a complexity in it which was not at first suspected. All investigators are agreed that in the presence of high concentrations of ammonia and of lactate ornithine exerts a catalytic effect, but this is only unequivocally true for small concentrations of

rnithine. High concentrations of ornithine depress rea formation. This is best seen in the work of Gornall nd Hunter (1943).

Bach, Crook and Williamson (1944) have made bservations on the effects of high concentrations of rnithine. They showed that if liver slices are subjected o several successive washings in oxygenated Ringer rginase is removed in rapidly diminishing amounts, ne washed cells retaining high arginase activity. The oluble arginase is presumably derived from the damaged ells of the slices. It can be shown to have the same ffinity for arginine as the arginase remaining in the ices. When large concentrations of ornithine are added o the washings (*ca.* 150 mg./100 ml.) arginase activity inhibited, and the same is true of the arginase activity f the washed slices. Nevertheless, when washed liver ices are incubated with ammonia and lactate, the effect f raising the ornithine concentration from 400 to 500 mg./100 ml. on urea formation is negligible: a milar change in ornithine concentration almost suppresses urea formation from added arginine. These elations are illustrated in Fig. 9 (overleaf). These bservations appear inconsistent with the idea that rginine is an intermediate in the formation of urea om ammonia and lactate in the presence of ornithine.

he kinetics of the ornithine effect:

ornall and Hunter (1943) have made the most extensive periments yet reported on the effects of various conntrations of ornithine and citrulline on the rate of nthesis of urea from ammonia. Fig. 10 is derived om their data and shows the relation between extra urea oduced in the presence of the added 'catalyst' (i.e. nithine or citrulline) and the concentration of 'catalyst'. oncentrations are plotted as logarithms of molarities order to accommodate the wide range of concentrans used. Gornall and Hunter themselves remark



CONCENTRATIONS OF ADDITIONS (mM)

Curve	Arginine	Ornithine	Lactate	Ammonia
1	0·43	—	—	—
2	0·43	12·12	—	—
3	—	3·03	1·79	1·87
4	—	12·12	1·79	1·87

FIG. 9

The rate of urea synthesis in washed liver slices in the presence of arginine (1), arginine plus excess ornithine (2), ammonium lactate with a little ornithine (3) and ammonium lactate plus excess ornithine (4). (*From Bach, Crook and Williamson*, 1944.)

that at some lower concentrations the extra urea production elicited by citrulline is significantly less than that elicited by an equimolar concentration of ornithine, but as the extra productions are not significantly different at higher concentrations, they regard these significant differences as artefacts. Fig. 10 suggests that it is erroneous to do so: the form of relation between compound added and effect is the same for both compounds,

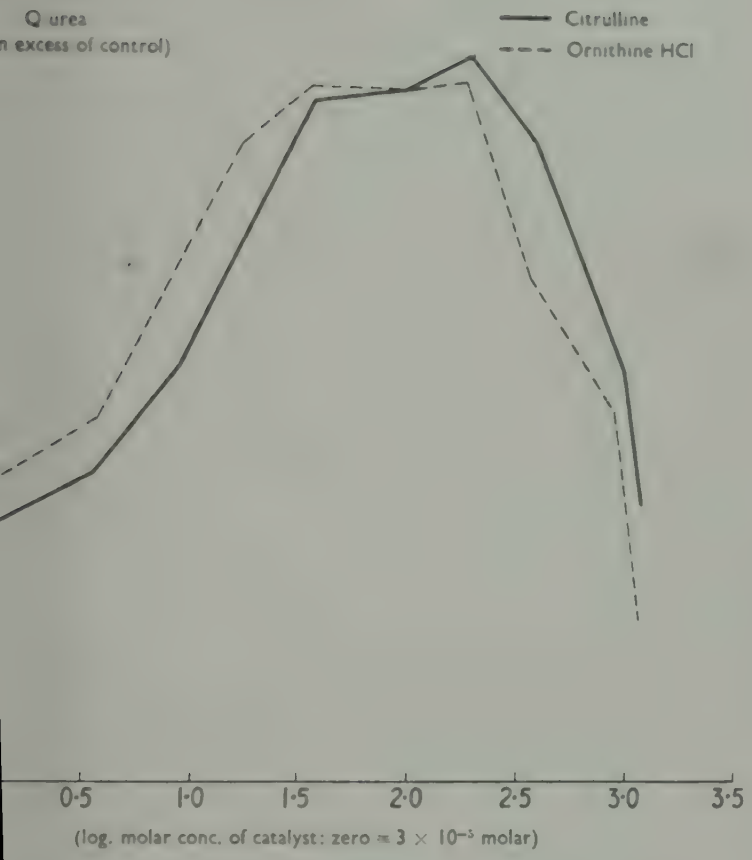


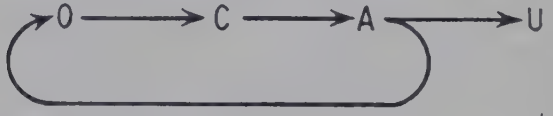
FIG. 10

he relation between the rate of extra urea formation pro-
d by the addition of ornithine or citrulline to liver slices,
e presence of ammonia and lactate, and the concentration
nithine or citrulline. (*Based on the data of Gornall and*
er, 1943.)

a maximum effect in the middle of the concentra-
range, and with the whole curve shifted bodily up
oncentration axis in the case of citrulline. It is
ly true that at every point in the range a given

concentration of citrulline produces the same effect as about half that concentration of ornithine. Although it is difficult to devise or apply a formal test of significance of the apparent systematic relative displacement of the two curves, there can be little doubt of its reality.

The importance of this relation is that it is the reverse of what should happen if the Krebs scheme is valid. If we write the Krebs reaction scheme in the form:



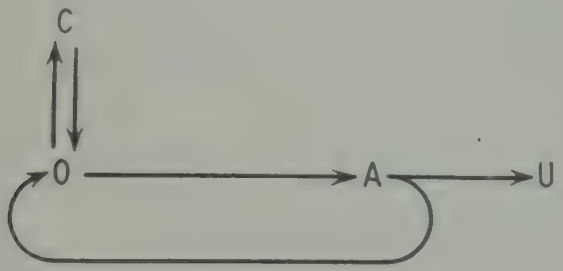
assuming that the concentrations of NH_3, CO_2 and H_2O are all in such excess that they can be considered to remain constant, we get the following expressions for the amounts of urea formed at a time t after adding ornithine or citrulline to give an initial molar concentration of M:

$$U_{orn} = qM(Kkt - k(1 - e^{-Kt}))$$
$$U_{cit} = qM(Kkt - (K - k)(1 - e^{-Kt}))$$

provided that each step in the reaction is proportional to the concentration of the ornithine, arginine or citrulline. This proviso is likely to be reasonably close to the truth at relatively low concentrations, but not to be so at high ones. The symbols q, K and k all refer to positive quantities, so that the difference $(U_{cit} - U_{orn})$ between the amounts of urea formed in the two cases, which is $qMK(1 - e^{-Kt})$, is also always positive. Thus, we expect, at least at low concentrations, that the citrulline 'catalytic' effect will always be greater than the ornithine effect. The Krebs scheme does not account

all for the phase in Fig. 10 in which increase in ncentration of 'catalyst' decreases the rate of urea oduction, but that is of no moment. The important ng is that the scheme also does not account for the ative effectiveness of citrulline and ornithine.

Gornall and Hunter (loc. cit.) found that a ureido- mpound, probably citrulline, accumulated when they cubated liver slices with ammonium lactate and nithine, but this does not help to establish the Krebs neme. Such a scheme as:



ould account for the conversion of ornithine to citrulline d for the lesser effectiveness of citrulline. Such a neme is consistent with the production of urea from ded citrulline, and with some degree of true catalytic fect of citrulline, but it does not account for Bach, rook and Williamson's observation that urea formation om ammonia in the presence of ornithine is not ected by change in ornithine concentration from 400 1,600 mg./100 ml., whereas urea formation from ginine is considerably inhibited. These ornithine con- ntrations correspond to the extreme right of Fig. 10. om the complex form of the relations between catalyst ncentration and rate of urea formation disclosed by is Figure it appears that the effects of ornithine at gh concentration on urea formation from ammonia clude much more than the simple catalytic effect. The

persistence of urea formation from ammonia in co ditions in which arginase activity is strongly inhibite suggests rather strongly that ornithine may participa in two different processes leading to urea formatio only one of which involves arginine formation. It ma well be that two ornithine cycles will have to be postu lated to account for urea formation, the less effectiv one involving citrulline and arginine.

The possible reversibility of liver arginase activity:

More space could well have been devoted to discussio of the complexities latent in the apparently simpl ornithine cycle, and one other basic point require attention before passing on to consider the work of thos who have accepted the ornithine cycle hypothesis an have attempted to determine the details of its operatio Krebs (1934) adduced in support of the scheme th argument concerning the distribution of hepatic arginas in different species which has already been set ou (p. 50). In this connection it is of interest to refe back to a further observation of Bach, Crook an Williamson (1944). They separated the arginase of live slices into the extracellular arginase of the washings an the intracellular arginase of the washed slices, an showed that the two had the same kinetics, suggestin that the enzymes were probably identical and that ther was no bar to the access of extracellular arginase t intracellular arginase. They then showed that, whils oxygen had no effect on the activity of the extracellula arginase it apparently considerably inhibited the intra cellular enzyme. It seems improbable that this effec could be a true inhibition of the enzyme. It is mor probable that in the presence of oxygen the cell is capabl of transforming arginine in some other fashion, e.g incorporation into peptide or protein, to a degree whic considerably reduces the amount available to th arginase.

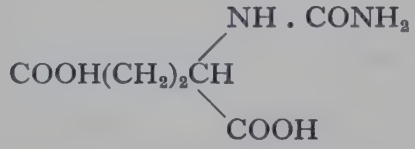
Modifications and extensions of the Krebs ornithine cycle: More recent work on urea synthesis has been largely in the direction of study of the capacity of isolated tissue preparations to catalyse the reactions postulated in the

glycocyamine

Krebs cycle. Borsook and Dubnoff (1941) showed that guanidinoacetic acid (glycocyamine) was formed when rat kidney slices were incubated with arginine and glycine in the presence of glutamine. Since they also observed that glycocyamine could be formed when citrulline was substituted for arginine, they supposed the tissue to be capable of catalysing the transformation of citrulline to arginine. This work was followed up by Cohen and Mayano (1946 a, b; 1947; 1948) and by Cohen and Morisolia (1948). They found that 'homogenates' of liver, suspensions in which the tissue has been so thoroughly broken up that no intact cells remain, will convert added citrulline to urea, provided that adequate amounts of adenosine triphosphate, inorganic phosphate, magnesium and cytochrome *c* are supplied. The glutamate used by Borsook, or some related compound, was necessary. Aspartic acid was less effective than glutamic acid, asparagine and glutamine were less effective still, and α-ketoglutaric acid even less effective. They interpret their results in terms of conversion of citrulline to arginine, but there can be no certainty that arginine is an intermediate in the urea formation they observe, and the requirements of their system are very different from those of Borsook and Dubnoff (1941). These authors found glutamate, aspartate, glutamine and α-ketoglutar-

ate plus ammonia to be roughly equally effective ac juvants to the conversion of citrulline to arginine in r kidney.

Cohen's homogenate system plus additions does n convert ornithine to urea, but if the magnesium omitted and potassium added, the system will readil convert ornithine to citrulline in the presence of adequa bicarbonate, CO_2, NH_3 and glutamate. The optim concentrations of glutamate, ammonia and ornithine a in the curious ratio of 20 : 2 : 1 (Cohen and Hayan 1947). A possible explanation of this is the finding b Cohen and Grisolia (1948) that the relatively unstabl carbamyl-L-glutamic acid enables citrulline productio to proceed two to three times as rapidly as in the pre sence of glutamate, CO_2 and NH_3.



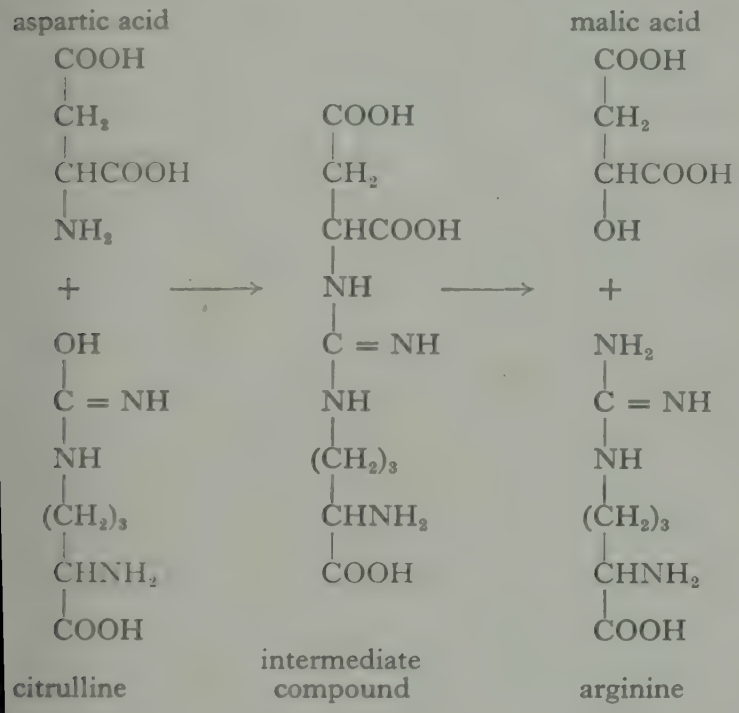
carbamyl-glutamic acid

Ratner and Pappas (1949 a, b) have made further con tributions which hardly clarify the issue. Their starting point is indicated by the following quotation (Ratne and Pappas, 1949a):

> 'It was not until Cohen and Hayano succeeded i demonstrating rapid arginine synthesis in live homogenates from glutamic acid and citrulline tha the transfer from an amino-acid rather than fro NH_3 was recognized as a main pathway in ure formation.'

They find that acetone powders of ox or rat live suitably fortified with sources of 'energy-rich' phosphat and a source of energy, will yield urea on addition o

aspartic acid and citrulline. They believe aspartate to be more effective than glutamate, and have demonstrated that aspartate is converted to malate. They have demonstrated that at least two steps are involved in the formation of urea, the first involving the formation of malate and the disappearance of citrulline, the second involving the formation of urea. They postulate the reactions shown:



and have isolated from reaction-mixtures an 'intermediate compound' as a barium salt (Ratner, 1949). The constitution of this compound does not appear to have been established, but the work has been interpreted on the assumption that it has the constitution shown above. As in Cohen's work, there is no indication that arginine is ever formed: in crude liver preparations it

might be difficult to demonstrate it because of the hig arginase activity, but in later work (Ratner and Petrac 1951) using fractionated preparations, arginase has bee deliberately added, without demonstration of the nece sity for its presence in order that urea should be forme

Hirs and Rittenberg (1950) have made a contributio to the subject, in a study of the fate of labelled putativ intermediates in urea synthesis added to washed live slices. High concentrations of all substrates were use and there is the usual uncertainty concerning th catalytic effect of citrulline. There is no doubt, howeve that when carbamyl-labelled citrulline is incubated wit ammonium chloride urea is formed in which the con centration of label is nearly 50 per cent. of that in th carbamyl-nitrogen. That is, in general, in these con ditions, urea synthesis occurs by a process to whic citrulline contributes one and ammonia presumably th other of the two nitrogen atoms per molecule. Thes workers also showed that when labelled aspartic aci was used in the presence of either ornithine or citrullin there was an incorporation of labelled nitrogen into th urea greater than could be accounted for by way o preliminary deamination of the aspartic acid. Never theless, ammonia appeared to be a more importan source of urea nitrogen than aspartic acid. Other experi ments indicated that glutamate, in the presence o aspartate, was utilized equally readily as it as a sourc of urea-N, that glutamine was relatively poorly used i the presence of ammonia and that glycine-N could b used to quite an appreciable extent without prio deamination. All these experiments on slices were mad in the presence of 10μM concentrations of all th various substrates, i.e. approx. 100 mg./100 ml. o ornithine and citrulline, approx. 20 mg./100 ml. o ammonia. There are no data for relative rates of ure formation, only for relative contributions of alternativ sources to whatever urea formation occurred.

Leuthardt (1938) has shown that liver slices will synthesize urea from added glutamine, in the absence of ammonia, and this reaction is catalysed by ornithine. Further, the activity of this system is not dependent on the presence of unphysiological concentrations of lactate or pyruvate. A physiologically plausible concentration of glucose is effective as an energy source. Krebs (1942) has confirmed Leuthardt and has compared the rate of urea formation in the presence of glutamine with that in the presence of glutamate and ammonia. The mean rates are identical in four comparisons, so that there is no clue to the relative positions of glutamine and glutamate in the synthesis. It is unfortunate that the only experiment of Hirs and Rittenberg with glutamine was made in the presence of ammonia, so that there is no indication whether glutamine can contribute directly. Hirs and Rittenberg do not envisage the possibility of more than one pathway leading to urea formation: their glutamine experiments were designed to test whether glutamine was an obligatory step in the conversion of ammonia to urea.

Every sort of discrepancy seems to have been manifest in these investigations of partial systems. There is little agreement between any two sets of workers on the relative significance of ammonia, glutamate, aspartate, glutamine. In such circumstances it is usually a good rule to believe that everyone has one foot on the right track and the other on a side-track. In other words, it seems highly probable that some one of the basic assumptions is misconceived, that the urea-producing system is more complicated than is generally assumed, and that all the judgments of relative importance of intermediates are true for partial systems, but that the liver is capable of forming urea by different methods, not necessarily of equal physiological importance.

It should certainly be kept in mind that much of this later work ought not to be taken to illuminate the

problem of the adequacy of the Krebs ornithine cycle account for the physiological production of urea. Ma of these studies have concerned themselves with t potentiality of tissue preparations to catalyse individu steps postulated for the cycle, the preparations bei such that they cannot usually catalyse more than o such step at once. In addition, conditions have be sought which favour the direction of transformati postulated for the cycle, without any guarantee that su conditions could obtain *in vivo*. Such partial studi provide no additional evidence to help to solve t crucial problems: why is citrulline less effective th ornithine, and is citrulline a chemical catalyst? In co trast to the position when McCance wrote his revie we now have a plethora of biochemical suggestions, b wholly inadequate pointers to their physiological bea ings.

We must take it for the present that these detail studies do not help to indicate whether deamination amino-acids is a chief process in protein catabolism.

The source of renal ammonia:

Too little is known of the processes of uric acid synthes in birds or other species in which it is a princip excretory product to yield useful indication of the for in which the nitrogen is transferred to protein. The o physiological process which might be supposed to thro some light on the transfer is the production of ammon by the kidney. Nash and Benedict (1921) showed th renal venous blood contains more ammonia than do renal arterial blood, making it clear that the kidn makes the ammonia it excretes. The kidney is also o of the two organs demonstrated by Krebs (1933) to b rich in enzymes capable of deaminating amino-acid (the other organ being the liver). So it is reasonable t expect that here, in the kidney, if anywhere other tha in the liver, evidence for a physiological process

deamination of free amino-acids might present itself. No such demonstration has proved possible, and it has recently been shown that there is good reason to suppose that the immediate source of the renal ammonia is blood glutamine (Harris, Roth and Harris, 1943).

Conclusion:

Thus the present picture of the general nature of protein catabolism is far from clear. The suggested means for transfer of amino-groups from protein to excretory products do not seem adequate to the task. Neither deamination of free amino-acids nor transamination to yield glutamic or aspartic acid appears able to account for the transfer, either on the score of number of amino-acids which can participate in the process, or on the score of activity of the transfer system. Glutamine is not satisfactory as a general intermediate, since it seems unlikely that the amide group can arise directly from free amino-acids.

It is undoubtedly true that isolated tissues can deaminate added free amino-acids, but this is not inconsistent with the view that deamination of free amino-acids is unlikely to prove a major process physiologically. It is well established, for instance, that added free amino-acids are converted by tissues into derivatives which are precipitated by trichloroacetic acid, which have been taken by some writers to be proteins. Such processes occur at high rates, and it is perfectly possible that these derivatives are more proximate sources of the products of deamination. Such a suggestion as this should not be considered too seriously at this stage of the discussion of protein metabolism. It is introduced to emphasize the point that the dilemma of protein catabolism almost certainly demands for its solution the introduction of hitherto unconsidered factors; some such advance as that made so dramatically by Krebs and Henseleit in 1932 is presumably around the corner.

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CHAPTER IV

GENERAL ASPECTS OF THE METABOLIS OF THE AMINO-ACIDS

Despite the doubts that have been expressed in earl chapters concerning the primacy of the amino-acids the natural form of protein currency, the major part current knowledge of processes which may constitu steps in normal protein metabolism is derived from t study of the metabolism of amino-acids. Before pr ceeding to an examination of what these studies c show, it is as well to be clear concerning some of t limitations of this sort of knowledge.

Although it is established that animals receiving appropriate mixture of amino-acids may grow as w as animals receiving a first-class protein, this does n detract from the conclusion reached in Chapter II th the primary step in normal protein metabolism is t anabolic step of protein synthesis, and one of the ain of this chapter will be to show how this can be so. O of the consequences of acceptance of this conclusion that the catabolic processes in protein metabolism a normally applied to the products of breakdown cellular protein. There is no *a priori* reason to suppo that these catabolic processes are purely hydrolytic un the stage of free amino-acid is reached, and there therefore no reason to suppose that the transformatio undergone by a relatively large amount of a single amin acid introduced into the organism as such are represent tive of the direction or magnitude of the transformatio undergone by the same amino-acid introduced as pa of a nutritionally balanced mixture of amino-acids or

component of a protein. Evidence that the course of metabolism of the amino-acid may be strikingly different s presented in this chapter.

On these grounds this book will not deal in any ystematic manner with the possible transformations of ndividual amino-acids, and in this chapter only such opics as general transformations supposedly undergone y amino-acids and the role of amino-acids in nutrition re discussed. However, these topics are of the first mportance for the understanding of the relation of mino-acids to protein metabolism: work of the ature of that discussed in this chapter provides some f the crucial evidence of the nature of protein meta-olism.

Pharmacological effects of amino-acids:

n interpreting the effects of the introduction of a large mount of an amino-acid into an animal, one has to ear in mind possible pharmacological effects. The harmacology of metabolism is in its infancy, but there s no doubt that many drugs can produce gross effects n metabolism and tissue composition, often unaccom-anied by, or long outlasting, any overt physical mani-estation. The effects of adrenaline on tissue glycogen nd on blood glucose, lactate, phosphate and α-amino-itrogen, the effects of tetrahydro-α-naphthylamine and f dinitrophenols on metabolic rate, the effects of echolyl, adrenaline and prostigmine on creatine nthesis (see Fisher and Wilhelmi, 1939) are sufficient lustrations.

One consequence of the occurrence of such effects that the accumulation or excretion of a metabolite llowing experimental administration of a chemical mpound ought not to be taken as evidence for the ansformation of the compound given into the meta-lite studied, even if the transformation is chemically lausible, until the possibility of a pharmacological

effect has been excluded as completely as possible. interesting instance of the pitfalls of plausibility afforded by the study of the effects of ingested meth purines on uric acid and allantoin excretion. Hess a Schmoll (1896) and Schittenhelm (1910) showed t ingested methylpurines brought about increases in u acid and allantoin excretion, and in both instances conclusion drawn was the chemically plausible one th the administered compounds had been demethylat Brugsch (1913) suggested that the increased excreti of uric acid or allantoin might be a consequence sympathetic stimulation by the methylpurines, a Dresel and Ullmann (1921) showed that in the rabbit t fivefold increase in rate of allantoin excretion which c be produced by the administration of methylpurines entirely abolished by bilateral section of the splanchn nerves. As Falta (1914) had already shown th adrenaline increases allantoin excretion in the dog, it more reasonable to suppose that methylpurines stimula allantoin excretion by a pharmacological process of son complexity than that they are demethylated and d graded to allantoin, despite the fact that in Dresel an Ullmann's experiments the amounts of extra allantoi excretion were of the order to be expected if larg fractions of the administered methylpurine had bee degraded.

'*Glucogenic*' *amino-acids:*

Similar considerations are of importance in interpretin the evidence for the conversion of amino-acids to glucos and glycogen. It is generally supposed that this occur and that the process is quantitative for glycine an alanine. Ringer and Lusk (1910) claimed this on th basis of the relation between the extra glucose and th extra nitrogen excreted by phlorrhizinized dogs follow ing the administration of these amino-acids. There no doubt that the correspondence between total extr

ucose and total extra nitrogen, taken over forty-eight ours, was consonant with quantitative conversion of mino-acids to glucose. But later work (Csonka, 1915; anney, 1915) suggested that the peak of extra glucose xcretion occurred before the peak of extra urea excre-on (see Fig. 3, p. 6), a relationship that would be ifficult to accept if the glucose were to be supposed be formed from what was left after its nitrogen had en split off as ammonia. Unfortunately, this appar-ntly insuperable difficulty seemed to be overcome by e demonstration by Nash and Benedict (1923) that hen glucose and urea were given together by mouth to phlorrhizinized dog the peak of glucose excretion receded that of urea excretion. Janney (loc. cit.) had conciled his findings with those of Ringer on the ounds of a '. . . greater permeability of the kidney for ucose . . .' and Nash and Benedict's findings appeared confirm this supposition.

hysiological factors in the study of 'glucogenic' amino-ids:

he compartmentation of physiology is such that consideration of Nash and Benedict's findings in the ght of more modern knowledge of intestinal absorption ems never to have been undertaken. It was only two ars later that Cori (1925) published the first evidence at glucose was absorbed from the intestine at a rate ry much in excess of that at which non-metabolized gars were absorbed. This is enough to suggest that ucose would enter the body much more rapidly than ea, and means that Nash and Benedict's results ow no light on the significance of the time relations served by Csonka and by Janney.

The intestinal factor is only one of the physiological tors that ought to be considered in this connection. appears probable, for instance, that whereas urea will netrate relatively rapidly into all the body water,

glucose will penetrate with considerable relative
culty into the intracellular water of muscle,
constitutes a large fraction of the body's intrace
water. This might be supposed to lead to s
proportionate rise in plasma urea concentration th
plasma glucose concentration. Further, urea is bel
to be passively reabsorbed to a considerable exte
the renal tubule, whereas, if the dose of phlorrhi
adequate, glucose should not be reabsorbed at all.
the glucose clearance should exceed the urea clear
Extra glucose, even if produced simultaneously
extra urea, should be excreted more rapidly on
counts, which makes the observed relation very dif
to reconcile with the hypothesis of glucose form
from the amino-acid administered.

Effect of amino-acids on liver glycogen:

These experiments on the phlorrhizinized dog are
however, the only experiments leading to the belief
the non-nitrogenous part of administered amino-
is converted to carbohydrate. There is a very
literature on the effects of injected amino-acids on
glycogen in the rat and the mouse. Schofield and L
(1947) have published one of the latest papers on
topic. One of the earliest investigations is that of B
Dunn and Hallmann (1935) which constitutes the
of a long series of studies. An important paper is
of MacKay, Wick and Carne (1940) in which the t
course of glycogen accumulation in the liver is illust
following administration of glucose, alanine or gly
The findings are shown in Fig. 11. The times of
accumulation for the amino-acids lie between ten
sixteen hours, and that for glucose is four to six h

However, just as with the methylpurines, the
evidence that some injected amino-acids can cau
secretion of adrenaline. Nord (1926 a and b), see Fig
has shown in rabbits that the injection of glycin

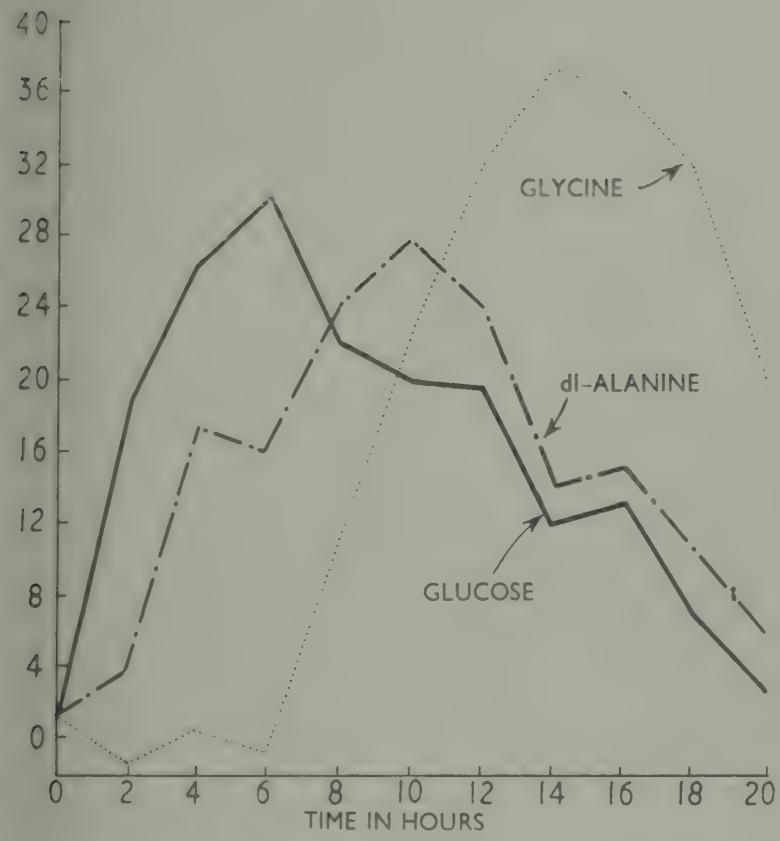


FIG. 11

The time-course of appearance of glycogen in the liver llowing the administration of amino-acids or of glucose to ts. In each instance 6 g. atoms of carbon per sq. decimetre dy surface in the form of glycine, alanine or glucose was ministered by stomach-tube (*MacKay, Wick and Carne,* 40).

utamic acid, both 'glucogenic' amino-acids, produces crease in blood sugar and blood lactate similar in time- urse to the increases produced by adrenaline, and milarly susceptible to abolition by ergotamine. He has so shown that the amino-acid effects are absent in

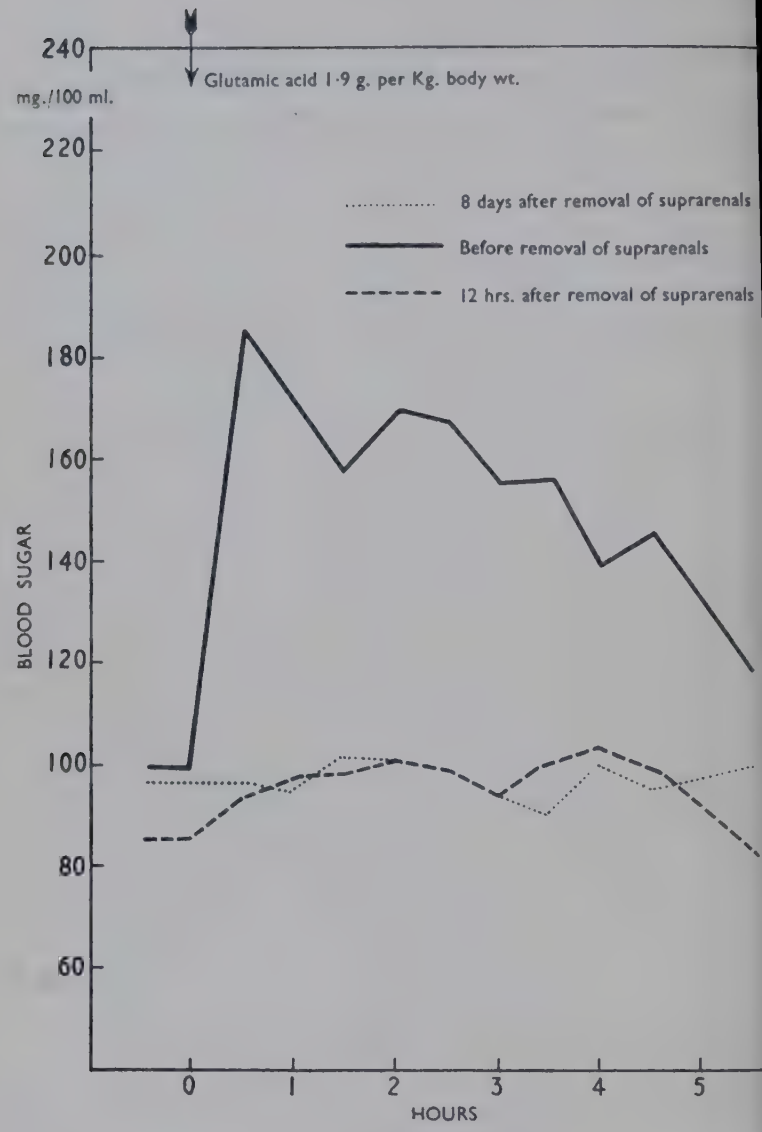


FIG. 12

The effect of administration of glutamic acid to a rabbit the blood sugar concentration. The effect is abolished suprarenalectomy. (*Folke Nord*, 1926.)

ne acutely adrenalectomized animal. Basiliou and Zell 1931) have obtained similar results but Lundsgaard 1930) failed to do so.

If the Nord effect occurs in dogs and rats it can ccount for the observed effects of 'glucogenic' amino-cids. The rise in blood sugar concentration produced ill necessarily result in increased glucose excretion by ne phlorrhizinized animal. It has been known for more nan twenty years (Sahyun and Luck, 1929) that drenaline produces a rise in liver glycogen content in abbits the time-course of which is much the same as ne time-course of liver glycogen accumulation observed y MacKay *et al.* in rats. This is illustrated in Fig. 13 verleaf) which also shows that the change in muscle lycogen does not account for the rise in liver glycogen. s we shall see later (Chap. VI) there are some lines f evidence that make it necessary to conclude that arbohydrate can be synthesized from *protein* in the ody, which is not the same thing as saying that it can be ynthesized from amino-acids. One line of evidence dis-ussed in that chapter suggests strongly that adrenaline an cause a secretion of adrenocorticotrophic hormone f the anterior pituitary which can in turn increase the ate of formation of carbohydrate from protein in the ver. Thus, even if administered single amino-acids are nemselves converted in any degree to carbohydrate, the otal effect on the glycogen of the liver will be com-ounded of three components, the effect of direct ansformation of the amino-acid, the effect of adrenaline n the mobilization of tissue glycogen and the effect of drenaline on gluconeogenesis from tissue protein.

otopic studies of glycogenesis from amino-acids:

he findings so far discussed demonstrate the difficulties simple interpretation of the effects of administration amino-acids on glucose excretion and on glycogen rmation. The complexity of the situation is such that

it is just as difficult to exclude the possibility of signi cant conversion of the administered amino-acid carbohydrate as it is to establish it. This is one of t occasions on which isotopic labelling techniques can g

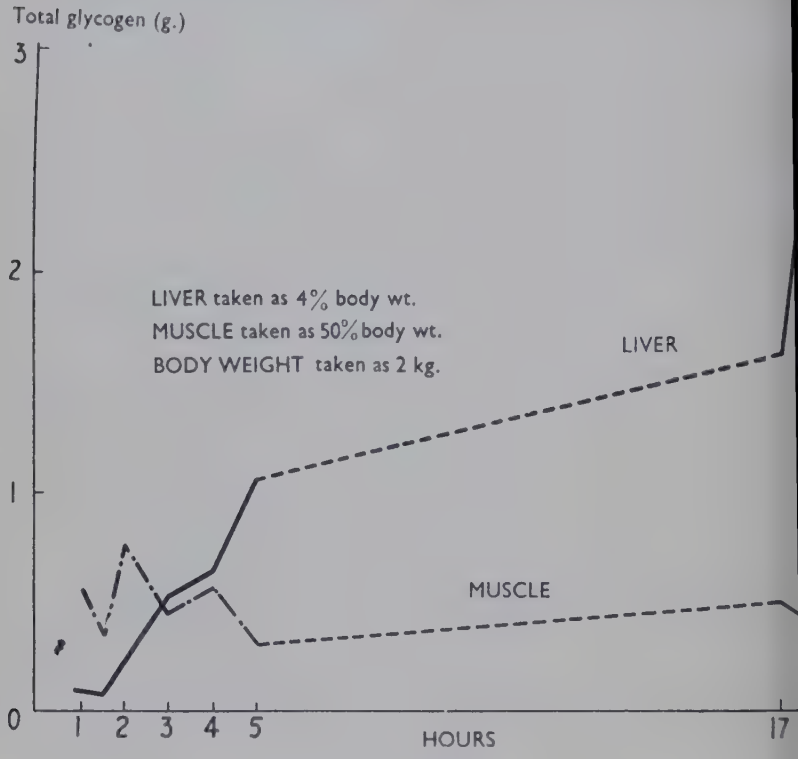


FIG. 13

The effect of injection of adrenaline on the total glycoge content of rabbit liver and skeletal muscle. (*Redrawn fro Sahyun and Luck*, 1929.)

a simple unequivocal answer. It has been shown b Olsen, Hemingway and Nier (1943) that when glycin labelled with ^{13}C is injected into rats and the liv removed at the time at which the characteristic increas in liver glycogen content is manifest the incorporation

^{13}C into the glycogen is so slight that not more than per cent. of the extra glycogen carbon can be considered to have been derived from the glycine. Since ome part of the glycine may be expected to have undergone non-specific changes in the period of hours between dministration and the death of the animal, and since ven so universal a metabolite as CO_2 contributes carbon hepatic glycogen, this finding is tantamount to a denial f direct transformation of glycine to glycogen.

A second isotopic study of great interest in this connection has been made by Gurin, Delluva and Wilson 947) who showed that when alanine labelled with ^{13}C as administered to phlorrhizinized rats extra glucose quivalent to 60–70 per cent. of the alanine was excreted ut contained only 1–5 per cent. of the administered otope. Since alanine gives rise to pyruvic acid, a ormal intermediate in carbohydrate metabolism, when is deaminated, the plausibility of conversion of alanine glucose in a stoichiometric manner is high, but the otopic data do not support the notion of such a conersion.

Thus it must be supposed that the evidence which as been interpreted in the past as support for the pothesis of transformation of amino-acids to carbodrate is in fact evidence for their stimulant action, in e dosages used, on the processes of carbohydrate metalism. Work in this field illustrates very forcibly the portance of the general proposition that the metabolic fects of a substance introduced into the body should t be interpreted as due to its own metabolic transrmation, however plausible the transformation may pear.

e fate of excess methionine:

a study of the metabolism of methionine in which e methylthiol group was labelled with ^{14}C Mackenzie, achele, Cross, Chandler and du Vigneaud (1950) fed

a synthetic diet containing twenty amino-acids includ
methionine for six to ten days, and then gave a sin
meal of a diet identical except in that its methion
was radiomethionine. When the diet contained 0·6
cent. methionine (which gives optimal growth) the r
of appearance of ^{14}C in the respiratory CO_2 rapi
settled down to a low steady value (Fig. 14). When
amount of methionine in the diet was doubled
initial rate of appearance of ^{14}C in the expired
was enormously increased, but the rate settled do
rapidly after the period of digestion and absorption
wards that observed on the 0·6 per cent. methioni
diet.

The difference between the initial rates of methioni
oxidation in these different circumstances was sho
not to be due to adaptation of enzymes to a high or l
level of methionine supply: when a single meal of t
1·2 per cent. methionine diet was fed to anim
habituated to the 0·6 per cent. methionine diet the sa
high rate of methionine oxidation was observed as wi
animals habituated to the high methionine diet.
approximately 6 per cent. of the lower dose and 25 p
cent. of the higher dose was oxidized in twenty-fo
hours, and as in both instances about 5 per cent. of t
^{14}C was recovered in urine and faeces these findin
mean that 11 per cent. of the smaller dose was lost
metabolized in twenty-four hours, but 38 per cent.
the additional methionine was lost. The dispari
between proportions oxidized is even more strikin
6 per cent. of the first 0·6 per cent. and 38 per cent.
the second are oxidized. It is abundantly clear that f
this amino-acid the fate of an excess and that of
nutritionally apt ration are strikingly different. One m
therefore justifiably feel that even the most unexce
tionally interpreted studies of the metabolic fates
excesses of single amino-acids may throw little light
protein metabolism.

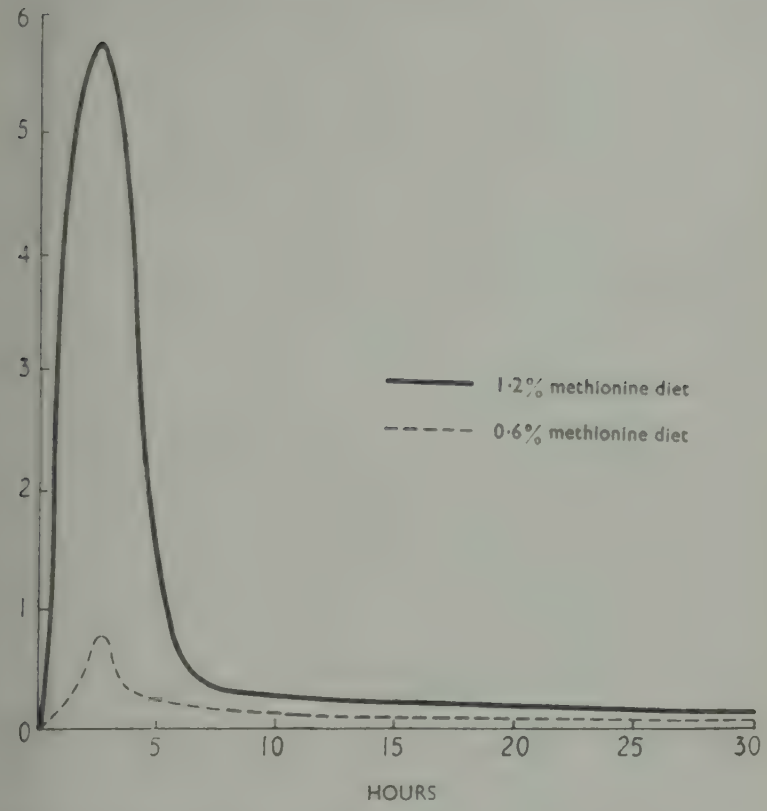


FIG. 14

The excretion of $^{14}CO_2$ in the expired air of rats following he administration of methyl-labelled methionine. The con-inuous line refers to the fate of a single dose of radio-methion-ne at a level of 1·2 per cent. of methionine in the diet, the nterrupted line to the fate of 0·6 per cent. of methionine in the iet. (*Mackenzie, Rachele, Cross, Chandler and du Vigneaud,* 950.)

Ketogenic' amino-acids:

n view of this conclusion, and because studies of the conversion' of amino-acids to acetoacetic acid and -hydroxybutyric acid are relatively few, little need be aid on the topic. Embden and Kalberlah (1906) found hat ketone bodies were liberated from perfused livers

by phenylalanine and by tyrosine. Edson (1935) fo
ketone body formation to occur in liver slices on addi
of phenylalanine and tyrosine. Since Butts and
colleagues (1938, 1941) found phenylalanine to ha
marked effect and tyrosine a slight effect on the accu
lation of glycogen in the liver, the evidence for
metabolic fate of these two amino-acids is confused.

Butts, Blunden and Dunn (1937) found that L
leucine increased ketone body excretion in rats, wh
they interpreted as conversion of the amino-acid
ketone body. No amino-acid other than these th
appears to have been clearly demonstrated to ca
ketone body liberation.

'Essential' amino-acids:

Physiological aspects of the metabolism of the ami
acids have been extensively studied in the course
determination of their nutritional significance. This
a form of study first undertaken by Osborne and Men
(1912) in the U.S. and by Ackroyd and Hopkins (19
in this country. Both these groups of workers show
that there were proteins which when fed as the s
source of protein failed to support growth, and that the
proteins could be rendered capable of supporting grow
if they were supplemented by some one amino-ac
Largely owing to the very great difficulty of determini
the amino-acid distribution in individual proteins, t
type of study, though yielding some results of gr
value, engendered much confusion and gave rise
many contradictions. The crucial advance was made
Rose, who achieved the outstanding feat of maintaini
good growth of rats on diets in which all but the triv
proportion of the nitrogen present in vitamin prepa
tions was supplied as purified amino-acids. In additi
to a major feat of chemical organization, this achiev
ment involved the discovery of a new amino-acid. Wh
the work began supplies of the naturally occurri

L-isomers of the amino-acids of the rigorous purity required for this work did not exist, so that a long series of difficult chemical preparations on a heroic scale preceded the biological work. When every known amino-acid thought likely to be necessary in rat nutrition had been assembled it was found that rats would not grow on a mixture containing generous supplies of all of them. However, Rose found that the addition of relatively small amounts of casein or fibrin digests to the mixture conferred growth-supporting properties on it, so that the daunting possibility that some one or other of the amino-acids was present in the mixture in toxic proportion could be set on one side. Fractionation of the casein digest soon revealed that the supplementary effect was complex, the ultimate solution being that isoleucine, a known amino-acid but an unsuspected essential, was one component, and threonine, a hitherto unknown amino-acid, was the other. This story, and the subsequent steps leading to the specification of the amino-acids essential for growth in the rat, is admirably presented by Rose (1938).

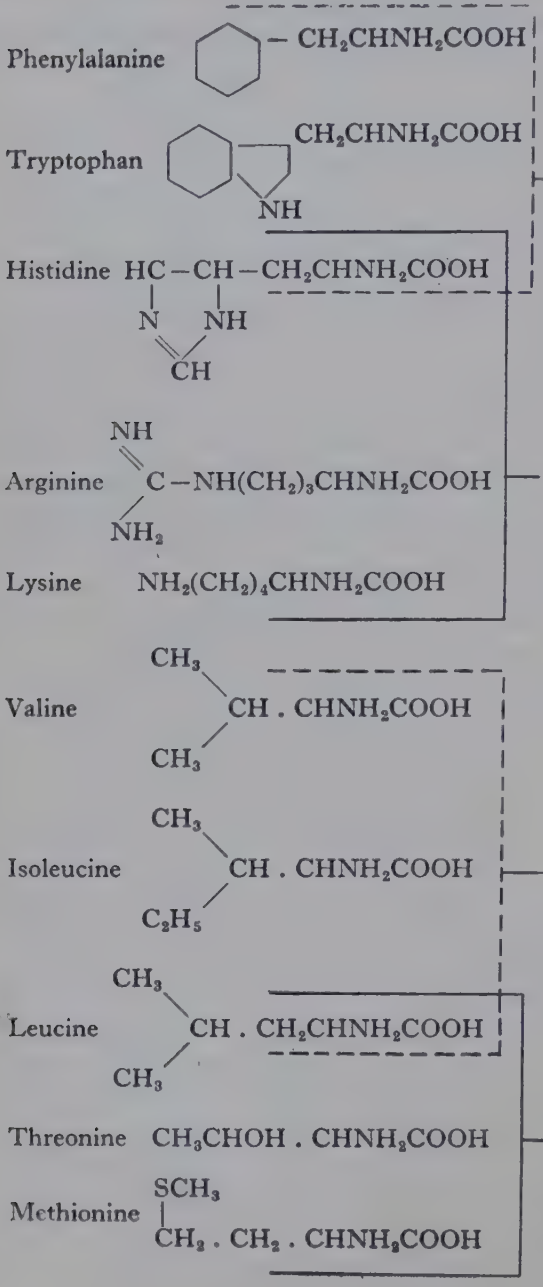
By removing amino-acids from the mixture one at a time Rose found that ten were essential for good growth. These ten are listed in Table 5 (overleaf) in a manner which helps to illustrate their structural relations.

Supplementary relations between amino-acids:

It has been known from the early days of the purified amino-acid work that the requirements of two of the essential amino-acids were diminished by providing chemically-related amino-acids which were not themselves essential for good growth. Thus there is growth when phenylalanine is supplied but no tyrosine, there is no growth when tyrosine is supplied but no phenylalanine, but growth can be restored in this instance by the addition of less than the full requirement of

TABLE 5

Rose's ten essential amino-acids grouped according to the chemical constitution



henylalanine. There is a similar relation between the ssential amino-acid methionine and the non-essential mino-acid cystine. All these statements refer to the t.

There is also evidence for some degree of partial pplementary relation in the rat between arginine and me other amino-acids. The position of arginine itself different from that of most of the essential amino-ids. Growth occurs when it is absent from the diet the rat, but it proceeds at a subnormal rate. This is conformity with the early finding of Scull and Rose 930) that the increment in body arginine in young rats own on a diet low in arginine is much in excess of the ginine intake in the experimental period. Young rats nthesize arginine, but not sufficiently rapidly to satisfy eir full requirements during the period of active owth.

Womack and Rose (1947) have taken advantage of this culiar position of arginine in studies in which the slow owth on diets containing all the essential amino-acids cept arginine has been compared with that following pplementation with arginine, proline, hydroxyproline, utamic acid or combinations of these. Proline or utamic acid was found to stimulate growth, and also creased the rate of growth obtained with arginine alone. omack and Rose suggested that these observations ant that arginine, proline and glutamic acid are utually interconvertible, but that all can be manu-tured from some other source or sources since moder-growth occurs in the absence of all of them from the t. On this view, the additional growth produced by pplementing an arginine-containing diet with proline glutamic acid is due to the sparing of arginine: wth requires the laying-down of all three amino-ds in the tissues, and the arginine may be supposed provide all three in the absence of the secondary pplements.

Non-specific supplementation of mixtures of essen amino-acids:

However, there are two other possible lines along wh these findings might be explained. The first is illu trated by a more recent study by Rose, Smith, Woma and Shane (1949). In this work weanling male rats we fed on a basal diet in which the only amino-acids su plied were the ten essentials, each being supplied at t level which is adequate for maximal growth when the is a liberal supply of all amino-acids, non-essential as w as essential. Growth occurs very slowly on this restrict diet (approx. 40 g. gain in twenty-eight days). The ra of gain may be more than doubled by supplementi the diet with ammonium citrate, ammonium acetate L-glutamic acid. Smaller but significant increases growth are produced by urea and by glycine suppl ments. The occurrence of growth on the essenti mixture alone suggests partial conversion of these non-essential amino-acids. (Note that 'essential' ha now necessarily become a technical term: the 'non essential' amino-acids are as essential to growth as th 'essential' ones, but it is not apparently necessary t supply to the animal each kind of non-essential amino acid; the animal can synthesize most or all of th optimal requirement of acids of this class from a non specific source of nitrogen, but the supply of tha nitrogen in some suitable form is as essential as th essential amino-acids.)

The validity of the interpretation of the weight gain in these experiments as true growth has been establishe by demonstrating a corresponding nitrogen retention The utilization of urea is surprising, and may be con nected with recent suggestions of the occurrence o urease in the gastric mucosa (Fitzgerald, 1946).

It follows from this work that the additional growt observed when arginine-containing diets were supple mented by proline or glutamic acid might have bee

ue in part to the provision of a non-specific source of itrogen available for conversion to any one of a large umber of amino-acids, of which arginine might be only ne.

he criterion: 'essential for growth':

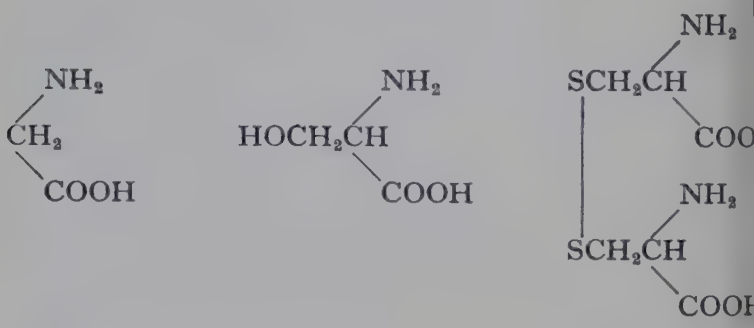
second and quite different possible way of accounting r the findings of Womack and Rose (1947) is suggested the very thorough study of amino-acid requirements the mouse which has recently been made by Maddy d Elvehjem (1949). In this work the criterion of lequacy of an amino-acid mixture is that it shall give owth equivalent to that given by a casein diet of the me nitrogen content. This is a far more definite iterion than that of 'good growth' and ought to prove ore sensitive. In Maddy and Elvehjem's hands it has oduced some very important results. It proved ecessary to supply six amino-acids to mice in addition Rose's ten essentials in order to obtain growth at the me rate as with the corresponding casein diet. These additional amino-acids were glycine, alanine, paragine, glutamic acid, serine and tyrosine.

The existence of this intermediate class of amino-ids need not be considered to extend the concept of sential amino-acids except in degree. For the rat the es of synthesis *in vivo* of Rose's essentials appear to entirely inadequate for growth, except in the instance arginine, but this does not mean that the other nine nnot be synthesized at all in the rat. Arginine is own to be synthesized, but at a rate markedly less an that necessary for good growth. The intermediate ss discovered by Maddy and Elvehjem in the mouse y be supposed to differ from arginine only in degree, to be synthesized at rates more closely approximating those necessary for maximal growth, but still not aching such rates.

These findings suggest that some part of the growth

effects found by Womack and Rose when mixture ten essentials were supplemented by glutamic aci proline may have been due to specific needs for t amino-acids.

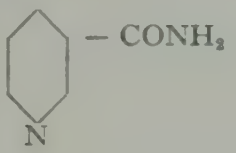
The importance of balance between amino-acids: The studies of Maddy and Elvehjem yielded ano set of results of great interest. In these experimen mixture of Rose's ten essentials in constant proport was supplemented by glutamic acid and glycine, the growth obtained compared with that obtained casein diets containing the same amount of nitro If the proportion of the mixture constituted by ei the essentials or the glutamate plus glycine rose ab a particular level, the growth rate fell. Addition of se to a mixture of the ten essentials plus glycine glutamate reduced growth rate by a third, whe addition of serine to a mixture of the fifteen o amino-acids found necessary to give as good growth casein definitely increased growth.



Amino-acids of secondary nutritional importance

Diminutions in growth rate produced by addition non-essential amino-acids are difficult to fit into a sim picture of specific requirements for 'essentials', howe

efined, and non-specific requirements for a topping-
o dose of nitrogen in one of a number of assimilable
rms. There are other pieces of evidence that a balance
ust be preserved between amino-acids in nutrition.
or instance, Allison, Anderson and Seeley (1947) and
rown and Allison (1947) have shown that whereas
oderate supplements of methionine increase the
nounts of nitrogen retained by rats receiving a casein
et, larger supplements may decrease the retention to
low the value obtained with casein alone. Such an
fect cannot be accounted for simply on the basis of
ilure to utilize the methionine: the supplement must
fluence the net availability of the casein nitrogen,
rectly or indirectly.



nicotinamide

Hankes, Henderson and Elvehjem (1949) have demon-
rated another intricate interdependence of amino-acid
quirements. Rats receiving a nicotinamide-free diet
ntaining 9 per cent. of casein supplemented by 0·2
r cent. cystine exhibit good growth. Addition of
078 per cent. L-threonine or 0·208 per cent. DL-
enylalanine cuts the growth rate to much less than
lf. Glycine will produce similar growth inhibition,
t a much higher concentration is required, in the
ion of 2 per cent. All three of these growth-inhibitory
ects disappear if the cystine supplement is removed
m the diet, but the substitution of methionine for
stine does not abolish them.
A point of interest is that growth inhibition equiva-
t to that produced by 0·208 per cent. DL-phenyl-
nine is produced by 0·104 per cent. D-phenylalanine,

and no inhibition was produced by the L-isomer. R
and Womack (1946) have stated that when libe
supplies of all non-essentials except tyrosine are inclu
in the diet, together with all essentials except phen
alanine, growth can be induced in rats equally well
adding D- or L-phenylalanine. Thus the three inhibit
are respectively an essential amino-acid (in Rose's sens
a non-natural substitute for an essential amino-acid a
a non-essential acid. The inhibitory activity of th
three substances depends on the presence in the d
of a non-essential amino-acid cystine and can be mask
by the addition to the cystine-containing diet of tryp
phan, an essential acid, or of nicotinamide, which
believed to be synthesized *in vivo* from tryptophan.

It is not possible to account for interactions of t
nature in the present state of knowledge: clearly t
metabolism of, or the metabolic effect of each of the
amino-acids is in some way interconnected with that
the others. The simplest though not necessarily t
adequate way of accounting for such interaction is
the assumption that the primary process in prote
metabolism is protein synthesis: if physiological catabo
processes involve the catabolism of protein, not
individual amino-acids, then any gross excess
deficiency in supply of any amino-acid will lead to
disturbance of pattern of supply which will leave so
amino-acid in excess. Such excess can be conceived
producing physiological disturbances by virtue of
own pharmacological properties or of those of
catabolites.

Temporal patterns in amino-acid requirements:

Direct evidence of the importance of pattern in amin
acid supply is afforded by work on the effects of spat
separation in the supply of inadequate proteins and
supplementary rations. The work of Geiger (194
sufficiently illustrates findings in this field. He f

ncomplete protein diets to rats together with supple-ents which were shown to render the diet capable of pporting growth when fed at the same time as the rotein. He then compared three types of feeding rocedure:

Type 1: Incomplete protein plus amino-acid supplement plus basal mixture (fat, carbohydrate, minerals, vitamins).

Type 2: Incomplete protein plus basal diet presented in one container, amino-acid plus basal diet presented in another, both containers available all the time to the animals.

Type 3: The two diets of Type 2 presented to the animals for alternate twelve-hour periods.

he same sort of results were obtained with three diff-ent incomplete proteins, deficient respectively in trypto-han, lysine, and methionine. (See Fig. 15, overleaf.) In ch instance Type I feeding gave good growth, Type 2 eding gave slight growth, Type 3 feeding gave no owth or some weight loss. In the instance of the ethionine-deficient diet it was formally proved that the ilure to grow in the Type 3 experiment was not due inadequate supplement intake: a group of Type 1 imals was given amounts of protein and supplement ual to the amounts that had been consumed by a oup of Type 3 animals on the previous day. The fference in growth between these groups was as great when paired feeding was not used. As food intake cords in the other experiments, in which paired ding was not used, showed that Type 2 and Type 3 ding regularly ensured 'adequate' supplies of supple-nt, the investigation as a whole must be interpreted meaning that protein metabolism involves the *ultaneous* metabolism of a mixture of amino-acids or ir condensation products.

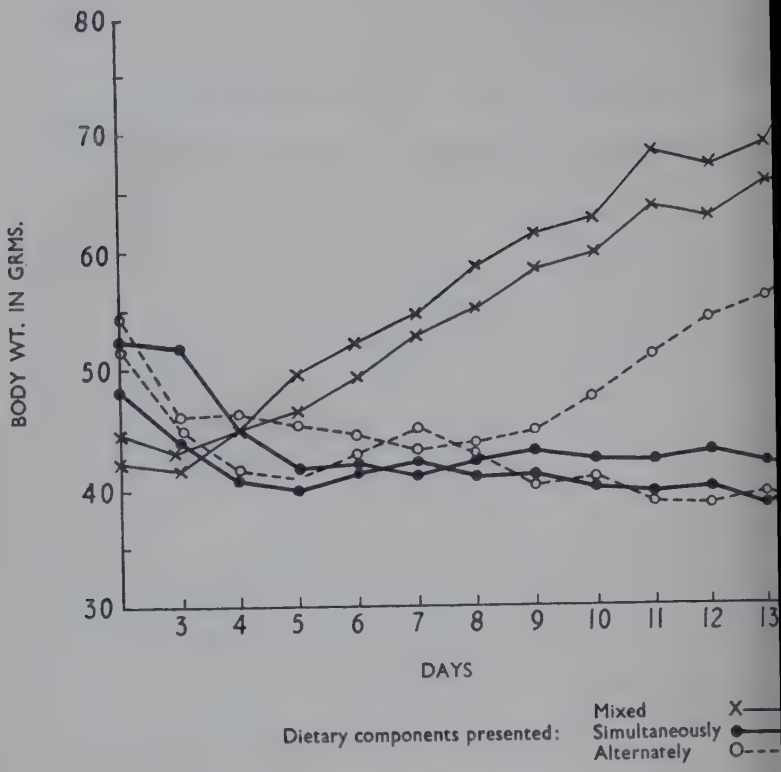


FIG. 15

The effect of separating an incomplete protein and its ami acid supplement in time or in space on the nutritive valu the combination. The growth curves shown are for receiving a mixture of oxidized casein + methionine, as 'incomplete protein' and tryptophan as the 'suppleme (*Geiger*, 1947.)

One possible defect in this study has been remed in a subsequent investigation (Geiger, 1948) in wh pairs of proteins have been found which will supp growth when fed simultaneously but will not suppor when fed successively. Pair-feeding was used in th experiments, and three different combinations g failure to grow when alternate feeding was used,

ood growth when simultaneous feeding was used. This isposes of the possibility that the earlier findings were n some way especially dependent on the fact that the upplement was in the form of a free amino-acid, which ight be regarded as an abnormal dietary constituent.

mino-acid requirements for growth in the chick:

here are two well-established major differences from ie rat. Arginine is essential for growth to occur at all Klose, Stokstad and Almquist, 1938) not merely for ptimal growth, as in the rat. In addition, glycine is sential for growth (Almquist and Grau, 1944). It is f interest that it has been claimed (Patton, 1939) that rge supplements of glycine are toxic to the chick.

Luckey, Moore, Elvehjem and Hart (1947) have made n extended study of amino-acid requirements for the nick which is of much the same nature as Maddy and lvehjem's (1949) study of mouse growth. Luckey *et al.* nd that casein supplemented with arginine, glycine and stine gives excellent growth. In one experiment this et caused growth at the rate of 6·7 g. per diem, but ubstitution of a mixture of twenty-two amino-acids for ie casein reduced the growth rate to 2·9 g. per diem, though the amino-acids were fed in the proportions which they are believed to occur in casein.

actors other than amino-acids determining the nutritive alue of protein:

uch findings as those of Luckey *et al.* suggest the ssibility that protein constituents other than the own amino-acids may play a part in determining the tritive value of protein. It is not impossible that there ay be still-undiscovered amino-acids, though the vance of analytical methods is rapidly reducing the elihood of this explanation. One other possible planation of such findings is suggested by the work Woolley (1945, 1946a, 1946b) who compared the

growth of rats receiving unhydrolysed casein with
of rats receiving acid-hydrolysed casein suppleme
with tryptophan. He found growth on the suppleme
acid hydrolysate to be much inferior. It could
restored to normal by a fraction from a tryptic dige
casein. The name strepogenin has been coined for
active principle of this digest. It has been shown
serylglycylglutamic acid accounts for some part
the growth-promoting activity of the strepog
fraction.

Maddy and Elvehjem (1949) showed that the addi
of a cellulose flour to diets containing amino-acid m
tures increased the rate of growth, but they were
able to detect any effect of addition of cellulose
casein diets. This sort of effect has been noted b
number of workers. Booth, Elvehjem and Hart (19
found that alfalfa meal or beet pulp added to a b
diet containing all known nutrients raised the gro
of guinea-pigs from 1·8 to 6·5 g./diem, confirming
earlier finding of Woolley and Sprince (1945)
roughage (cellulose flour or cellophane) could incre
growth in this species. Davis and Briggs (1947, 19
have found similar effects in chickens. Some of
data seem to suggest that factors other than mere b
contribute to this sort of effect in some species, bu
requires to be kept in mind that the consistency of
diet as well as its chemical composition may have so
significant influence on its nutritive value: the co
parison of growth on an adequate protein diet and
one in which an amino-acid mixture is substituted
protein may not provide as good a criterion of the a
quacy of the amino-acid mixture as would appear
first sight.

D-amino-acids in nutrition:

The ability of the D-amino-acids to replace their
isomers in nutrition should throw light on the ea

TABLE 6

lability of optical isomers and of derivatives of amino-acids for the promotion of growth

	CHICK		MOUSE		RAT			
	Amino-acid	*Ref.*	*Amino-acid*	*Ref.*	*Amino-acid*	*Keto-acid*	*Hydroxy-acid*	*Ref.*
lalanine			D=L	1	D=L			16, 17
tophan	D<L	21	D<L	18	D=L	+	O	2, 14, 19 5, 12 11
line			D<L	18	D<L	+	+	6 10 7, 10
e			L	18	L		O	3 15
e	L	9	L	1	L			16
ucine	L	9	L	1	L	+		16
ne	D=L	9	L	1	L	+		16
nine	L	8	L	1	L			20
ionine			D=L	1	D=L			13, 14

L signifies that both promote growth equally well.
<L signifies that the D-isomer is less effective than the L-
signifies that the L-isomer alone promotes growth.
signifies that a non-amino-compound can replace an essential amino-acid.
signifies that a non-amino-compound cannot replace an essential amino-acid.

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auer and Berg (1943)
erg (1934)
erg (1936)
erg and Potgeiter (1932)
erg, Rose and Marvel (1929–30)
onrad and Berg (1937)
ox and Rose (1926)
rau (1949)
rau and Peterson (1946)
arrow and Sherwin (1926)
ckson (1927)

12. Jackson (1929)
13. Jackson and Block (1932)
14. Jackson and Block (1938)
15. McGinty, Lewis and Marvel (1924–25)
16. Rose (1938)
17. Rose and Womack (1946)
18. Totter and Berg (1939)
19. du Vigneaud, Sealock and van Etten (1932)
20. West and Carter (1938)
21. Wilkening and Schweigert (1947)

stages of amino-acid catabolism. If the D-isom be utilized for growth readily there is presumal active enzyme with L-isomer specificity whic aminate the keto-acid. Such an enzyme must b posed to be capable of deamination—but wheth immediate substrate for such amino-group trans the free acid or a peptide cannot be determined. D-isomer cannot be utilized for growth there ar fortunately several possibilities. There may h enzyme capable of deaminating it at an adequate there may be no enzyme capable of reaminating the L-isomer or the residue left after deaminatio be metabolized so rapidly that the maximum ava yield of L-isomer is negligible.

Data on the relative nutritive values of the isom the 'essential' acids to the rat, mouse and chic collected in Table 6. It is of interest that the c ponding keto-acids can be substituted for the L-isc of leucine, isoleucine, histidine and tryptophan i rat (Rose, 1938; Harrow and Sherwin, 1926; Jac 1929; Berg, Rose and Marvell, 1929–30), but that in one of these instances is the D-isomer nutritio equivalent to the L-isomer. An instance not incl in Table 6 is that of cystine. Only the L-isom cystine will spare methionine in the rat (du Vign Dorfmann and Loring, 1932) although both isome methionine are equally available. Since, however, now known that cystine can be formed from methi *in vivo* by a process in which only the sulphur is t ferred and in which the α-carbon and its asymm substituents are not concerned, this relation need give rise to difficulty.

On the whole, the data of Table 6 do not contr appreciably to knowledge of amino-acid metabo Too many possible ways are open to account for failure or success of a non-natural isomer to subst for its natural fellow.

Amino-acid requirements for nitrogen balance:

In the adult rat (in which growth still continues, but very slowly in proportion to body mass) it is found that the three basic amino-acids histidine, arginine and lysine are unnecessary for the maintenance of nitrogen balance. In the dog only arginine can be dispensed with (Madden, Carter, Kattus, Miller and Whipple, 1943). In man histidine and arginine can be dispensed with (Rose, 1947; Holt, Albanese *et al.*, 1942). But Madden *et al.* (loc. cit.) found that although N balance was maintained in their dogs the rate of regeneration of plasma protein occurred at a subnormal rate. Holt *et al.* (loc. cit.) found that in man the arginine diets which maintained nitrogen balance for ten days produced a great impairment of spermatogenesis.

In contrast to these instances in which protein-synthetic processes in particular organs appear to fail despite apparent satisfaction of the net requirements of the organism there are instances of successful maintenance of local synthetic activities in conditions in which the organism is starved of protein. Daft, Robscheit-Robins and Whipple (1933) showed that anaemic dogs can make considerable amounts of haemoglobin during starvation and Davis and Whipple (1919) showed that liver regeneration occurs in dogs after experimental induction of necrosis even when the dogs are fed on a diet of sugar alone.

Miescher's (1874) example of the disappearance of muscle tissue and the large increase in testicular mass in the migrating salmon, which is said not to feed during migration, also comes into this category.

It seems that maintenance of nitrogen balance in short-term experiments may not be a good criterion of the adequacy of an amino-acid mixture to serve the needs of the organism. If the organ least satisfactorily supplied is small, as is the testis, or manifests a delayed effect on function, as does the erythropoietic tissue of

the bone marrow, a considerable local negative nitrogen balance or a complete cessation of function might well not manifest itself externally during a nitrogen balance experiment of any readily practicable duration. The problem raised here is one which has frequently been discussed. McCollum and Simmonds (1925) advocated protracted feeding experiments for the proper demonstration of the adequacy of a protein diet, for reasons of much the kind stated. Mitchell and Hamilton (1929) put the case very well for short-term experiments, which have the advantage of relative uniformity of conditions, of reference to a defined epoch in the subject animal's life, and of lending themselves with far greater readiness to the procurement of quantitative measures of nutritive value.

Amino-acid excretion on different diets:

The last aspect of amino-acid metabolism which calls for attention in this chapter is that of effect of variation in the adequacy of the protein in the diet on the rats and nature of amino-acid excretion in the urine. Pearce, Sauberlich and Baumann (1947) investigated the excretion of bound and free amino-acid by mice fed either casein, an oxidized casein in which the tryptophan and methionine had been destroyed, or oxidized casein supplemented with one or both of tryptophan and methionine. The oxidized casein supported growth when it was supplemented with both these amino-acids. Table 7 gives the results of this work.

Free amino-acid excretion is profoundly altered by the adequacy or otherwise of the diet. This is not merely a matter of the fate of ingested supplements of amino-acid. In Table 7 the least excretion of free amino-acid among the amino-acid supplemented groups occurs in the group receiving the greatest supplement, and Sauberlich, Pearce and Baumann (1948) have shown that free amino-acid excretion varies even in animals on

TABLE 7

The effect of feeding complete, incomplete and supplemented proteins on the urinary excretion by mice of free and bound amino-nitrogen

Protein	*Supplement*	*Amino-acid excreted as per cent. of amino-acid ingested*	
		Free	*Bound*
Casein	—	2·9	3·6
Oxidized casein	Methionine	16·2	2·8
Oxidized casein	Tryptophan	25·0	3·2
Oxidized casein	Methionine + tryptophan	3·5	2·9

adequate protein diets according to the relative efficacy of the protein to support growth. These findings are illustrated in Table 8. Together with the previous

TABLE 8

The relation between the ability of proteins to support growth and the extent to which their amino-nitrogen is excreted into the urine by mice

Protein fed	*Weight of mice*		*Gain*	*Per cent. of ingested amino-acid excreted*
	Initial	*At four weeks*		
Casein	13·2 g.	19·9 g.	6·7 g.	3·4
Egg albumin	13·2	20·8	7·6	2·7
Fibrin	13·4	23·2	9·8	1·5
Lactalbumin	13·2	23·2	10·0	1·0

Four animals in each group

findings they support in a fresh fashion the thesis that protein metabolism is fundamentally that of a restricted mixture of amino-acids, amino-acids additional to such a mixture suffering a fate different from that of the adequate mixture.

The chances that the mixture made available by the diet at any one time will be exactly adequate are very small, so that the metabolism of dietary protein must at all times be supposed to consist of two parts, the metabolism of the mixture adequate for protein synthesis, and the metabolism of the residues of 'unemployable' amino-acids. Thus when large amounts of single amino-acids are fed the major catabolic transformations which they undergo are 'physiological' in the sense that they may be experienced by some part of the normal ration of the same amino-acids, but they are not 'physiological' in the sense of constituting the only or the major sorts of transformation of the normal ration of these amino-acids. However, if it is to be supposed that the amino-acid excretions of excess amino-acids which have been observed by Pearce *et al.* (loc. cit.) and by Sauberlich *et al.* (loc. cit.) may be accompanied by oxidative breakdown such as that observed by McKenzie, Rachele *et al.* (1950), then these processes may often contribute quite considerably to the totality of nitrogen metabolism. That is, depending on the instantaneous adequacy of the protein ration, there may be expected to be a varying, but often quite considerable 'exogenous' component of protein metabolism.

Criteria of adequacy of protein diets for young animals: The growth criterion has proved valuable in practice and has yielded great contributions to knowledge. But Maddy and Elvehjem's (1949) studies as well as those of Luckey *et al.* (1947) suggest that the more rigorous the criterion is made the fewer the amino-acids are which can be called non-essential. The work of Sauber-

h *et al.* (1948), quoted above, indicates that there are oteins which can give considerably better growth in ce than does casein. None of Maddy and Elvehjem's c. cit.) diets gave better growth than casein. It is still possible to say whether this must be taken to imply at more than sixteen amino-acids must be supplied yield 'optimal' growth in the mouse.

In addition to this uncertainty, there still remains the estion whether a diet is adequate for a young animal nen it supports maximal growth. Measurements of owth rate depend on the increase in the massive tissues the body. Failure of growth or differentiation of a nall organ, or development to an extent or in a direction at may be biologically unfavourable at a later epoch uld conceivably accompany increase in mass at a rmal rate. An instance which might possibly be explained along these lines is to be found in the comparison the findings of Hartoft and Best (1949) and of Sobin d Landis (1947), both of whom subjected weanling ts to brief periods of acute choline deficiency, and then aintained the survivors. Hartoft and Best kept the rvivors on a good diet on which they grew approximately normally, and observed the subsequent development of hypertension and renal damage in a very high oportion of their animals. Sobin and Landis kept their imals on a diet containing suboptimal quantities of oline, on which they grew at subnormal rates. No gns of hypertension were observed. This comparison one between strains of rat as well as between treatents, so that it cannot be taken as more than suggestive. Another instance is to be found in the relation between owth rate and longevity. Many workers have commented on this point, but perhaps the most striking idence is that of McCay (1933) who compared the e-spans of fully fed rats attaining maximum weight 4 months with those of underfed rats attaining maxium weight in 18–24 months. One only of 36 fully

fed rats survived after 37 months (1,100 days), compared with 21 of 70 underfed rats. The mean life span of fully fed rats was just on 500 days, that of underfed rats just on 800 days. It appears that the bigger is not in all ways the better in the matter of diet.

Conclusion:

The discussion in this chapter leads to the conclusion that although the animal organism is capable of metabolizing individual amino-acids fed in excess, and although small excesses of amino-acids may be normally available to undergo such transformations, yet the major processes of protein metabolism involve the amino-acids in concert, and cannot be inferred from the fates of excesses of individual amino-acids administered experimentally.

The work on the nutritional value of administered amino-acid mixtures indicates that growth can occur to a considerable extent on mixtures containing a very restricted number of amino-acids, which in turn signifies a considerable capacity of the organism for interconversion of amino-acids. This work does not, however, signify that such conversions are an indispensable or even a normal part of amino-acid metabolism in physiological circumstances.

The positive findings of most significance discussed in this chapter are those indicating the concert of metabolism in the protein field. It would be rash to elevate to the rank of a general principle the rule that metabolic pathways usually involve apparently irrelevant conjugations. It took many years to establish that the major pathway of carbohydrate metabolism involves conjugation with phosphate, and it is still not clear what role phosphatide formation plays in the catabolism of fat. But it is entirely plausible that the metabolism of amino-acids should be profoundly influenced by the accident of conjugation with other amino-acids. If the normal

ths of metabolism are those of the conjugated acids
en the many studies of the metabolism of excesses
single amino-acids are of little relevance to normal
otein metabolism, and we are justified in leaving them
others to discuss.

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CHAPTER V

HE USE OF ISOTOPES IN THE STUDY OF PROTEIN METABOLISM

ıe purpose of this chapter is to discuss the contribution isotopic studies to protein metabolism. It should be ar from what has been said in previous chapters that is difficult, and possibly unprofitable, to attempt to ess the results of experiments in which a large ıount of a single labelled amino-acid has been adminis-ed to an animal already receiving a ration on which animal may be presumed to be in nitrogenous uilibrium. There are many such studies, which could tifiably be discussed under the heading of the tabolism of the amino-acids. It is questionable ether they should have a place in a discussion of otein metabolism, and most of them will not be dis-ssed here.

There are considerations of a technical character ich must be borne in mind in interpreting isotopic dies. Three sets of isotopes are generally useful in study of protein metabolism: (1) the nitrogen topes, ^{14}N and ^{15}N, the latter constituting only 7 per cent. of all nitrogen atoms in nature; (2) the bon isotopes ^{12}C, ^{13}C and ^{14}C; ^{13}C constitutes 0·011 cent. of natural carbon atoms, and the third isotope , which is radioactive, was originally described as an ificial product, but is now known to occur in nature, parently being produced by bombardment of organic tter by cosmic rays; (3) the hydrogen isotopes, usually ed hydrogen, deuterium and tritium, and having pective atomic weights of 1, 2 and 3. Hydrogen and

deuterium occur in nature, deuterium constituting 0·02 per cent. of natural 'hydrogen' atoms. Tritium is an artificial radioactive isotope.

Technical considerations in the measurement of radio-isotopes:

Although there are numerous important details in methods for determining the abundance of radio-isotopes in a preparation of a chemical compound, the principle is simple for those radio-elements which emit penetrating particles as part of the process of atomic decay, since the count of penetrating particles emitted per unit time is proportional to the number of radio-active atoms present in the sample examined. By means of a Geiger-Muller tube, which produces an electrical pulse each time a particle of sufficient energy enters it, and of electronic apparatus to enable these pulses to operate a mechanical counter, estimates can be made of the concentration of the radio-element in any preparation, whatever the state of chemical combination of the element. This is a great convenience, and there is a further advantage that the technique is as a general rule sufficiently sensitive to permit the determination of very small amounts of radioactive isotope.

However, accurate measurement presents considerable technical difficulties. The emitted particles leave the specimen in all directions. They may rebound from collisions with layers of the specimen interposed between their atoms of origin and the Geiger-Muller tube (self-absorption). They may begin their careers by moving away from the detector tube and rebound from more distant specimen layers or the specimen container (back scattering). In any event only a fraction of them can enter the detector tube, the fraction being dependent on the precise spatial relations of specimen and detector as well as on self-absorption and back scattering.

Further than this, the proportionality between number

radioactive atoms present and number of particles
nitted per unit time is a statistical one, since the dis-
tegration of any one atom is uninfluenced by the state
others. Enumerated over a sufficiently large number
atoms and a sufficiently long time interval, the
oportion of atoms emitting particles per unit time will
ffer infinitesimally from a value p, just in the way
at if sufficient coins are tossed independently the
oportion showing heads will differ trivially from one-
lf. If only two coins were tossed it would not be
rprising if the proportion of heads were any of all,
e-half or none, and less extreme variations would be
cepted without surprise as the results of tossing other
nall numbers of coins. For this sort of reason a large
mber of counts must be recorded for reliable measure-
ent of radio-isotopes. Additional trouble is furnished
the 'background count'. It is difficult to shield a
tector tube from penetrating particles of the cosmic
diations. This is a good reason for using a relatively
gh concentration of the radio-isotope, the total count
en being increased more by increase in counting rate
an by increase in counting time. But there is a limit
the permissible increase in counting rate. Though
e detector tube requires little time to recover from the
fects of one particle before it can detect the next, yet
does require some time, and there will be a counting
te at which the probability of penetration of two
rticles into the detector within its resolution time will
come too high to be negligible. Similarly, the
echanical recorder will take a finite time to make each
vance and to be ready to accept another pulse.
though the devices interposed between detector and
echanical recorder usually serve to make it possible
record only hundreds of particles mechanically, the
solution time of the mechanical recorder is so much
eater than that of the detector that the mechanical
corder usually sets a limit on counting rate. If this

recorder has a resolving time of 0·1 sec., then the average interval between the pulses it is expected to count must be about 0·5 sec. if the loss due to coincidence is to be less than 1 per cent. It follows from these considerations that estimations of radio-isotopes are as much subject to error as other types of chemical estimation and that the findings of single experiments carrying no estimate of error should be treated with all normal caution.

Biological considerations in the use of radio-isotopes: It has to be kept in mind that radiations and particles emitted by radio-isotopes may have deleterious effects on tissues. It is thought that the mean intensities of tissue bombardment produced by the low concentrations of isotope which are usually used are unlikely to be deleterious, judging by the experience accumulated in X-ray and radium therapy. But, if a chemical compound tends to be transformed by a particular enzyme, current teaching suggests that it must form an enzyme-substrate compound with that enzyme, the substrate combining with the enzyme at a specific location. At any one time, then, in the cell in which this transformation takes place, a relatively high proportion of radioactive substrate molecules will be in a particularly close relation to one type of enzyme molecule: it is possible that this particular type of enzyme molecule may be exposed to a high enough concentration of radiation to alter the concentration of active enzyme molecules of this species during the experiment. Since the pattern of metabolism is determined by the relative concentrations of enzymes, this could be a point of importance.

Another point to be borne in mind is that the emission of radiation from a particular atom signals the transformation of that atom into another of different chemical properties. This is unlikely to be important in the instance of radio-carbon, since half its atoms will still

rvive undegraded after 5,000 years, but the appearance strange new chemical compounds in the experimental stem is a factor which ought to be kept in mind in periments with rapidly decaying radio-elements.

easurement of non-radioactive isotopes:

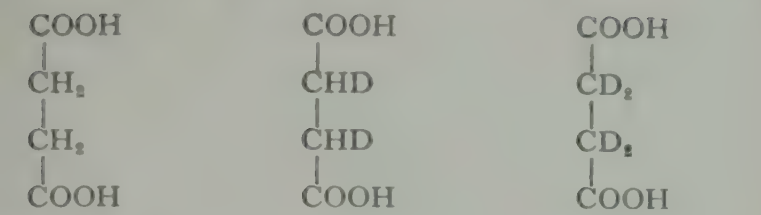
ie most generally applicable method is based on con-rsion of the element to a gaseous form of combination, . ammonia or carbon dioxide, and the introduction the gas at very low pressure into a vessel in which it bombarded by a high velocity electron beam. The s which are produced by this bombardment are ised to move forward as a beam by an accelerating ctric field, and this beam then passes between the es of a magnet which deflects it. The extent of flection in a given magnetic field depends on the ocity and mass of the ions. The low pressure vessel, hin which acceleration and deflection occur, contains o a metal chamber with a slot in it, placed so that the t faces the deflected ion beam. Ions of any one mass n be caused to pass through the slot into the chamber altering the strength of the accelerating field, and e current carried by these ions can be measured. The io of the currents carried by two different classes of n is the ratio of their relative abundance in the gas roduced into the apparatus.

When, for instance, the gas introduced is ammonia a ajor fraction of the ions produced will be in the forms $^{14}N^{14}N$), ($^{14}N^{15}N$) and ($^{15}N^{15}N$). If q is the proportion ^{14}N atoms in the nitrogen introduced and p ($=1-q$) the proportion of ^{15}N atoms, then the above three ecies of ion will be present in the relative proportions q^2, pq and p^2. Since, even in rich sources of ^{15}N, is not often over 0·05, p^2 is very small. The usual ocedure is to determine the relative abundance of $^{15}N^{14}N$ and $^{14}N^{15}N$, which gives directly the ratio pq, i.e. $q\,p$ or, since $q = 1 - p$, $(1 - p)/p$. That is,

provided the recording apparatus can measure the ratio q/p accurately, the value of p can be determined. There is no great difficulty about selecting the right accelerating voltage. Progressive change of voltage in the right region will yield progressive rise and fall again of current through the collector. The point at which the current is to be measured is that at which it reaches a maximum. There are technical difficulties in measuring large q/p ratios, e.g. those of the order of 200–300 which would be obtained for atmospheric nitrogen. Even when these are surmounted there is still need to plan for a value of p which is large compared with that found in nature.

The question of the chemical equivalence of isotopes:

The necessity to use fairly high ratios of rarer to commoner isotopes depends on the following considerations. Isotopes are defined as species of atom having the same chemical properties but different atomic weights, but this definition is not quite accurate. The chemical properties of isotopes are qualitatively the same, but not necessarily quantitatively so. Atoms of different mass will exhibit differences of volume and inertia, so that the detailed spatial configurations and frequences of vibration of the atoms of a molecule containing a heavier isotope may differ from the corresponding properties of a molecule containing a lighter isotope of the same element. Such differences may be difficult or impossible to detect when the atomic weights are large, but they are certainly not negligible when the two isotopes have atomic weights of 1 and 2, as in the instance of hydrogen and deuterium, and they may not be entirely negligible in the region of atomic weights of 12–15, i.e. in the carbon-nitrogen region. Thorn (1949) has recently shown that the rate of oxidation of succinic acid by succinoxidase is very considerably diminished by substitution of deuterium to give either dideuterosuccinate or tetradeuterosuccinate.



succinic acid | dideutero–succinic acid | tetradeutero–succinic acid

The rates of oxidation of these two compounds are ectively 70 and 40 per cent. of the rate of oxidation he deuterium-free compound. If similar relations l for all dehydrogenation processes, and if the rogen or deuterium could be eliminated from the nism with equal ease as water or deuterium oxide, organism might be expected to come to contain a ificantly higher proportion of deuterium than occurs ne physical environment. If, on the other hand, the erential rate of oxidation found by Thorn for sub- ted succinic acids is true only for specific dehydro- ation processes, the relative abundance of deuterium different organic compounds isolated from living nisms should differ markedly. Accurate determina- of very small proportions of deuterium is difficult, data on this point do not seem to be available.

imilar considerations ought to apply, though to a ch less marked extent, to the instances of carbon and ogen. Some estimate of the differential rates of tion of compounds containing different isotopes is e found in a note by Bigeleisen (1949). He suggests the differential effects are likely to be negligible for ns of the size of those of carbon and nitrogen. wever, his conclusions apply to single-stage reactions it remains possible that in the complex multi-stage dy-state systems of the living cell there may be a ificant differential accumulation of isotopes. In this nection one of the earliest papers on the use of ^{15}N

has an interest. Schoenheimer and Rittenberg (1939) examined one sample of casein and twelve samples of amino-acids isolated from natural sources. The casein and ten of the amino-acid samples showed a higher relative abundance of ^{15}N than did atmospheric nitrogen, measured in the same apparatus. (The most convenient measure of this relative abundance is 'atoms per cent. excess'. The normal abundance of ^{15}N is taken as 0·368 atoms ^{15}N per 100 total atoms of nitrogen. A sample in which the abundance was 0·468 atoms ^{15}N per 100 would be reported as possessing 0·100 atoms per cent. excess.) In the amino-acid samples referred to above the relative abundance of ^{15}N lay between 0·000 (two instances) and 0·010 atoms per cent. excess. In particular, four samples of arginine gave values ranging from 0·007 to 0·010, the mean being 0·0825 ± 0·0006. The fact that these measurements were made by direct comparison with atmospheric nitrogen in the same apparatus precludes the possibility of accounting for these excess abundances as due to systematic error. It is equally difficult to ascribe them to random error in the instance of arginine, where the observations themselves show that the mean atoms per cent. excess is significantly greater than zero. Schoenheimer and Rittenberg (loc. cit.) stated that 'It cannot yet be decided whether the slightly higher values found . . . demonstrate a real increase in the ^{15}N concentrations. . . .' Further evidence on this point still seems to be lacking at the present day, and one should therefore treat all small values of relative abundance with caution.

Fundamental experiments with ^{15}N-labelled amino-acids: Some of the fundamental results yielded by the isotopic labelling methods are, however, quite independent of these reservations. For instance, when labelled glycine or leucine was added to the diet of adult rats in nitrogen equilibrium on a 16 per cent. casein diet in amounts

esponding to 25 mg. N per diem for three days, 55 per cent. of the ^{15}N could be recovered from the due after trichloroacetic acid extraction of the tissues tner, Rittenberg, Keston and Schoenheimer, 1940; oenheimer, Ratner and Rittenberg, 1939). This due is usually described as protein, but there is no on for supposing that it is as simple as that: apart n the possibilities of association of free amino-acids peptides with protein precipitates, there are tri-roacetic acid insoluble peptides. The remainder of the can be accounted for in the faeces (2–3 per cent.), urine (30–40 per cent.) and in the trichloroacetic extract of the tissues (10 per cent.). The low faecal tent suggests effective absorption. Much the same ure is found when glutamic acid, aspartic acid, sine or lysine is fed (see Vennesland, 1948). The result is then that when a labelled amino-acid is as an addition to an already generous protein ration n adequate protein the predominant fate of it is rporation into the trichloroacetic acid insoluble tion of the tissues, which is probably largely protein. his incorporation of labelled nitrogen into tissue tein' occurs at different rates in different tissues, the t rapid uptake being by the abdominal viscera, with etal muscle much slower and skin slower still, and e detailed study of the 'protein' fraction of particular ans shows that although incorporation of the fed no-acid as such into tissue 'protein' is the pre-inant contributor to the process of uptake of ^{15}N, ^{15}N does also make its appearance in other amino-s. This is shown in Table 9 (overleaf). Unfortun-y there appears to be no single experiment in which extent of this broadcasting process has been studied in nimal in which an isotopically-labelled acid has been stituted for, rather than added to, the normal ration he amino-acid in an adequate diet. In the experi-nts just described the labelled constituent was

TABLE 9

Concentrations of ^{15}N in amino-acids isolated from tissue proteins of animals fed with labelled amino-acids (concentrations expressed as per cent. of the concentration of isotope in the amino-acid fed)

Amino-acid isolated	*Amino-acid fed*					
	Glycine		*L-leucine*		*D-leucine*	
	Liver	*Carcass*	*Liver*	*Carcass*	*Liver*	*Carcass*
Glutamic acid	0·89	0·27	1·85	0·89	1·33	0·17
Aspartic acid	0·73	0·20	1·16	0·70	1·03	0·24
Arginine	0·78	0·09	0·89	0·24	1·19	—
Glycine	**8·86**	**1·04**	0·76	—	0·55	0·14
Tyrosine	0·47	0·13	0·50	0·20	—	0·11
Lysine	—	—	0·06	—	—	—
Leucine	—	—	**7·92**	**1·90**	**0·77**	**0·25**

The glycine data are from: Ratner, Rittenberg, Keston and Schoenheimer (1940).

The L-leucine data are from: Schoenheimer, Ratner and Rittenberg (1939).

The D-leucine data are from: Ratner, Schoenheimer and Rittenberg (1940).

More extensive data are tabulated by: Vennesland (1948).

regularly added as an extra to diets in which the animals were apparently already in nitrogen equilibrium. It is obvious that in precisely those conditions there is maximum opportunity of observing secondary reactions not characteristic of a steady state of adequate protein nutrition: the experiments are made in conditions analogous to those in which Mackenzie, Rachele, Cross, Chandler and du Vigneaud (1950) observed excessive oxidation of excess methionine (see p. 89). The point which has been particularly emphasized by the early workers with ^{15}N, that isotopic studies demonstrate the

raordinarily broadcast nature of protein metabolic ctions, is one of which too much can easily be made, ne forgets the tendentious nature of the experiments. is at least equally striking that in these unfavourable nditions of luxus supply of an amino-acid the chief e which it undergoes is incorporation into the tissues the form in which it is fed.

cursor-product relationships:

ype of investigation of great potential importance in tein metabolism is the investigation of the speed of ction in a steady state system. For instance, if the r maintains a more or less constant protein content, crude chemical analysis can throw any light on the es of synthetic and degradative processes which are stantly occurring. Isotopic labelling makes it possi- to study such a system, and the fact of acquisition and subsequent loss of labelled molecules of tissue tein is easy to establish, as we have already seen. t the detailed interpretation of the findings is far from y, as may be seen by reference to an important paper Zilversmit, Enteman, Fishler and Chaikoff (1943), o treat the relatively simple instance of the turnover phospholipid in various organs.

Two major points are made in this paper. The first hat it is impossible to specify quantitatively the rate replacement of a metabolic product in a steady state tem unless the time-course of the intensity of labelling its immediate precursor is known. The rate of orporation of label into the product is dependent on o factors: (i) the rate of formation of new molecules d (ii) the proportion of new molecules bearing the el, and this second factor is given by the time-course the intensity of labelling of the precursor.

The second point is that a particular sort of relation ound to obtain between the time-course of labelling the precursor and that of labelling of the product.

This is of importance in the whole field of turnover rate determinations, but it is also of importance in all instances of attempts to use relative degrees of labelling of pairs of compounds as evidence for their metabolic relation.

An instance of the particular relation to be expected is illustrated in Fig. 16. It may be put verbally in this way. In any instance in which the intensity of labelling of the precursor successively rises and falls during an experiment, the intensity of labelling of the product will also rise and fall in a fashion which lags behind the precursor, and the two intensities of labelling will be identical only at the moment at which the product is most intensely labelled. A steady-state system is one in which numbers of product molecules formed per unit time equals number disappearing. The proportion of labelled molecules can therefore only rise so long as the proportion of precursor molecules which are labelled is higher than the proportion of product molecules which are labelled. The intensity of labelling of product can only be steady when precursor and product have the same intensity of labelling and the degree of labelling of product must begin to fall as soon as the intensity of labelling of precursor falls below that of product.

Thus, for the most intimately related pairs of labelled compounds identity of intensity of labelling is to be expected to be the exception rather than the rule in this type of experiment. Only in experiments in which a constant supply of labelled precursor is ensured can identity or non-identity of degree of labelling be used as evidence of metabolic relationship: in such experiments the intensity of labelling of the product will become equal to that of the precursor some time after the intensity of labelling of the precursor has become constant.

It will be seen that if one wishes to study the dynamic state of the body constituents dynamic and not static criteria of interrelations in metabolism must be em-

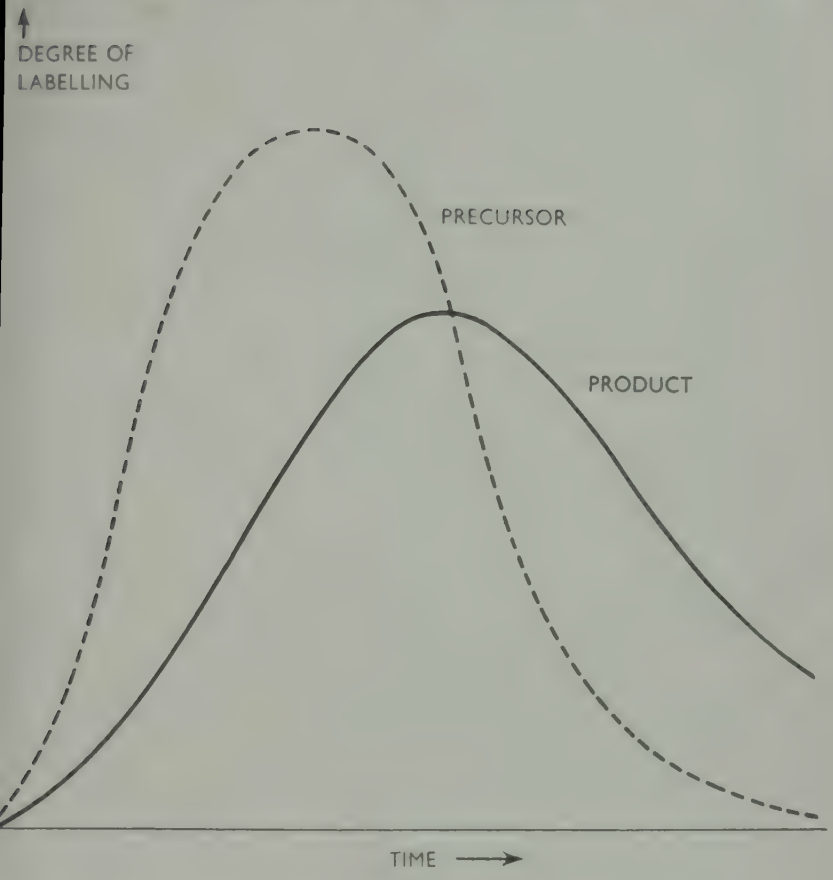


FIG. 16

This figure shows the type of relation likely to be found between the degree of labelling of a temporarily-labelled immediate precursor of a normal metabolite and the degree of labelling of the metabolite itself, the degree of labelling of the two being equal only at the instant at which the product metabolite is maximally labelled.

ployed. Thus, in the instance of protein, unless one is content to beg the question and to assume that when a particular amino-acid is fed it is to be regarded as the immediate precursor of all the various proteins formed in all the organs exhibiting detectable protein synthesis,

it would seem impossible in the present state of knowledge to provide more than approximate solutions to the turnover problem.

Approximate measurement of tissue protein turnover: Schoenheimer, Ratner and Rittenberg (1939) administered to adult male rats leucine labelled with ^{15}N in the amino-group and with deuterium in the side-chain. Since leucine is known to be absolutely essential for growth it was taken that the rat could not synthesize the side-chain and that the deuterium would provide a better index of incorporation of this amino-acid into tissue protein than would the ^{15}N, which could conceivably exchange readily with ^{14}N from general body supplies. The mean deuterium content of the leucine present in the diet was 1·8 atoms deuterium per 100 total hydrogen plus deuterium atoms (1·8 atoms deuterium per cent.). The leucine isolated from the liver contained 0·44 atoms deuterium per cent., so that at least 0·44/1·8 or 0·24 of unlabelled liver leucine had been replaced by labelled leucine. The corresponding estimate for carcass protein was 0·067 replaced in three days.

The diets used in these experiments are normal except for the 23 mg. of leucine nitrogen added each day. But the diet without this addition is already adequate, and contains an amount of leucine nitrogen equal to the amount of labelled leucine added. It is to be doubted, therefore, if the final mixture can be described as normal: it is comparable with that with which Rachele *et al.* (1950) observed excessive rates of oxidation of added methionine. The estimate which can be derived from these observations of the extent to which administered leucine can be incorporated into tissue proteins must be regarded as a minimum estimate.

Schoenheimer, Ratner and Rittenberg (1939) developed an isotope dilution technique for the determination of the total leucine of the rat. An amount of labelled

ucine containing a known amount of ^{15}N is added to
ie hydrolysate of the tissue proteins and uniformly
ncorporated in it. A sample of leucine is isolated from
ie hydrolysate and purified. From the ratio of the
^{15}N content of this leucine to that of the labelled leucine
dded, and from the known amount of labelled leucine
dded the total leucine of the tissue hydrolysate is
irectly determined.

This technique gives 183 milliequivalents as the
ucine content of an adult male rat. If it is assumed
iat the fraction of the leucine in the total body protein
hich is replaced in three days is the same as the fraction
eplaced in carcass protein (which contains 70 per cent.
f the ^{15}N recovered in protein) then the total protein
ucine replaced is 0·067 of 183 milliequivalents, i.e. 12
illiequivalents. The total leucine intake is calculated
s 39 milliequivalents, so just on 30 per cent. will be
stimated, on this basis, to have been incorporated into
hat is here described as tissue protein.

The authors note that this is a minimal value because
ie calculation assumes that none of the deuterium has
een removed from the carbon skeleton. Since it has
lso to be considered that the animal probably had twice
s much leucine available as it had of those amino-acids
ith which it required to condense the leucine to form
rotein, this estimate suggests that normally the incor-
oration of dietary amino-acids into tissue proteins must
e quantitatively far the most important immediate fate
f these compounds. From the point of view of estima-
on of turnover rates directly, however, this work does
ot contribute a great deal.

Shemin and Rittenberg (1944) have studied the dis-
ppearance of ^{15}N-labelled glycine and of total protein
^{5}N from tissues after a period of feeding ^{15}N-glycine.
everal points arise here which are illustrated by Figs. 17
nd 18 reproduced from this paper. Fig. 17, demon-
trating the time-course of disappearance of the isotope

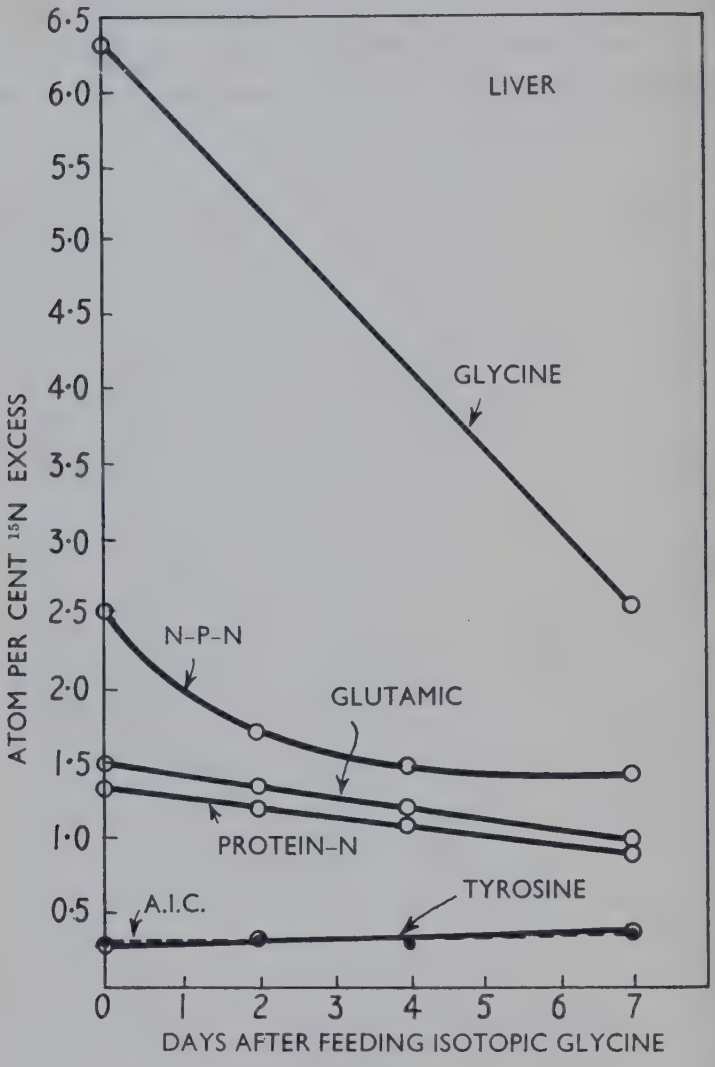


FIG. 17

The degree of labelling with ^{15}N of constituents of rat liver in the period commencing at the end of a three-day period of feeding of labelled glycine. (*Shemin and Rittenberg*, 1944.)

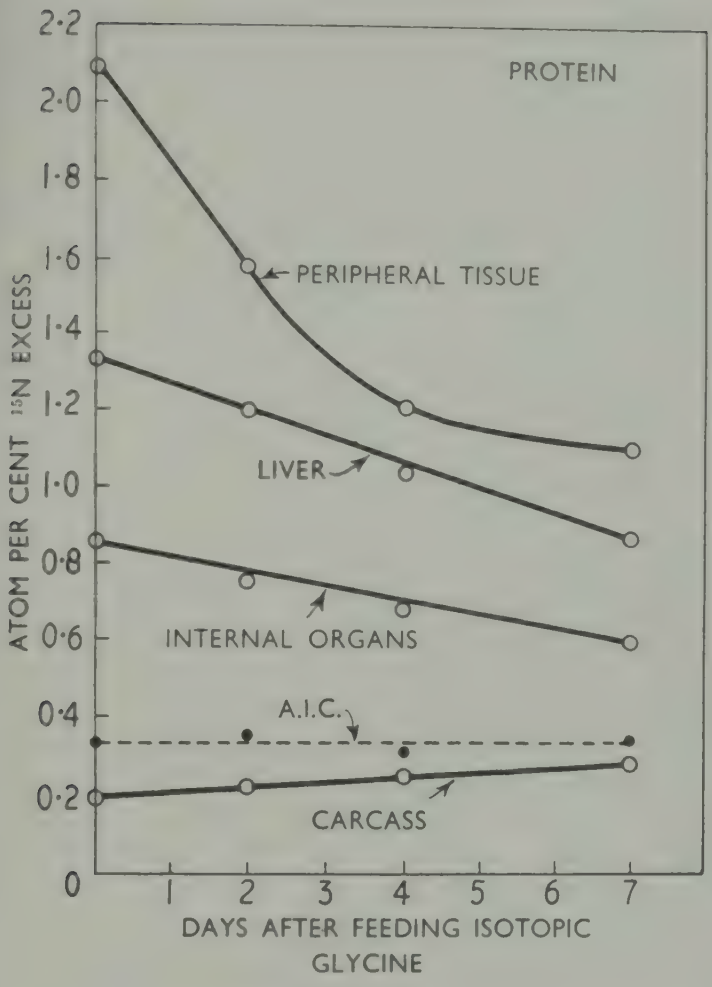


FIG. 18

The degree of labelling with ^{15}N of the protein fraction of different organs of the rat in the period commencing at the end of a three-day period of feeding of labelled glycine. (*Shemin and Rittenberg*, 1944.)

from glycine and from other amino-acids of the liver shows again that the preponderant fate of the amino acid fed is to be incorporated into the tissue protein as such. The estimate of turnover of liver protein on the basis of the isotope content of its glycine unfortunately has to be based on two experimental points, which cannot give a particularly reliable estimate of the position of the disappearance curve of glycine-^{15}N, and give no notion of its shape. Shemin and Rittenberg compute a half-life, on the basis of the two values given, on the assumption that the glycine available for replacement of liver protein glycine has the ^{15}N-content of carcass protein glycine, and on the assumption that the liver protein ^{15}N-content will ultimately fall to the 'average isotope content' (shown as A.I.C. on the figures) of the tissue as a whole. This is taken to be 0·35 atoms per cent excess, which they find in the total nitrogen of all the groups of tissues examined.

Unfortunately, there is no indication that the fall in glycine represents a simple turnover, amenable to such calculations, and Fig. 18, showing the time-course of total protein ^{15}N of the liver, indicates a linear and not an exponential fall, so that the use of the term half-life is extremely misleading.

If in a particular organ there were a steady turnover of a constant amount of protein, then a given constant fraction of the protein molecules would be replaced every day. If it could be assumed that breakdown were random, i.e. that the probability of breakdown of a molecule was independent of its time of survival, then a constant fraction of labelled molecules present at the beginning of each day should be lost by the end of that day. If half the labelled molecules have been lost in a given period, then in the next period of equal length half those present at the beginning of this second period will be lost. The line showing the decrease of isotope concentration as a function of time will follow the

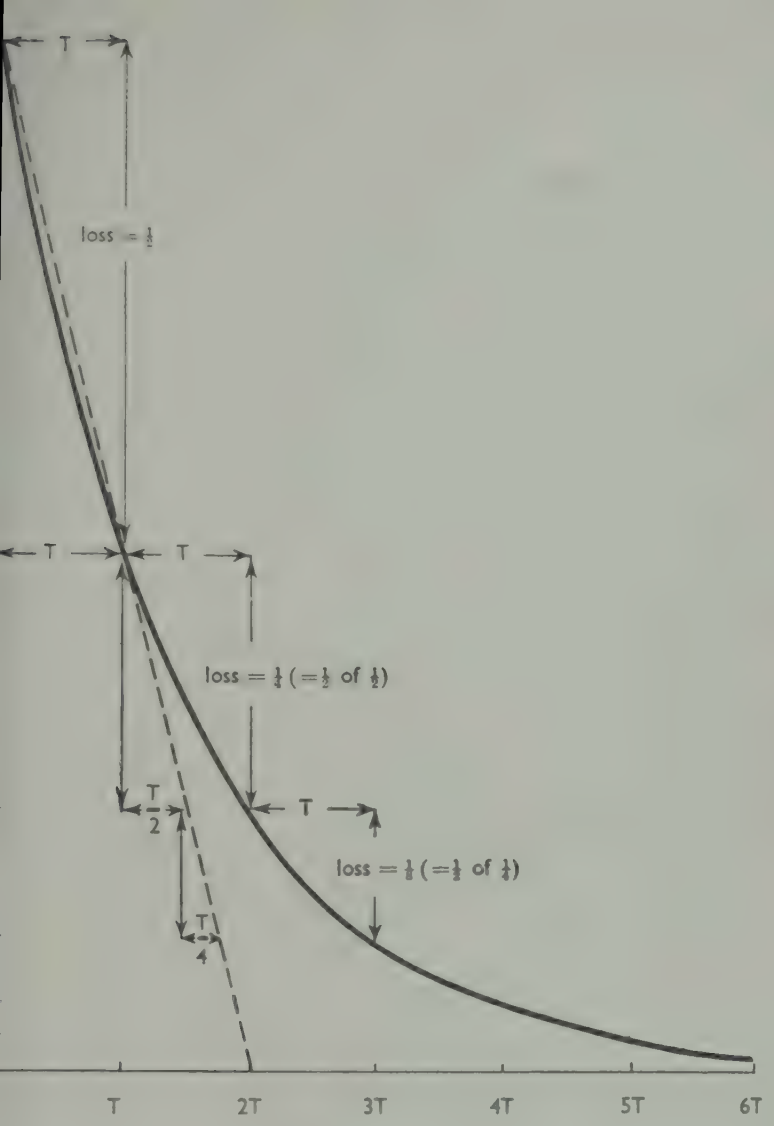


FIG. 19

A graphical demonstration of the inapplicability of the con-
ot of 'half-life' to linear decay of degree of labelling. The
ve is that of an exponential decay, the degree of labelling at
end of every period of length T being half as great as at the
ginning of the period. This is therefore the curve of decay
resenting a constant half-life of length T. The straight line
the character that the times of successive halving of the
gree of labelling are themselves successively halved: no
aracteristic half-life can be attached to such a type of decay.

K

course of the curve in Fig. 19, and the time T in whic half the initial isotope is lost is then legitimately calle the 'half-life', and represents the time in which half th protein of the tissue is replaced by fresh molecules. Th straight line in Fig. 19 represents a relation in whic successive replacements of total protein take progre sively shorter times, i.e. a relation in which turnov becomes progressively faster as time goes on.

It seems, therefore, that the course of exchange protein molecules leading to the progressive alteratio of labelling of protein nitrogen which is shown in Fig. 1 is not due to simple replacement at more or less com parable rates of all or most species of protein molecul and that, although one could speak of a 'half-life' as th time at which half the original isotope had disappeare from the liver protein, this figure should not be inte preted as being the time of replacement of half th protein molecules of the liver.

One other indication of the complexity of protei metabolism which is afforded by this work of Shemi and Rittenberg is given in Fig. 18, which shows th time-course of labelling of carcass protein following th period of glycine feeding. It will be seen that the exte of labelling of carcass protein is increasing during th period in which that of the liver is decreasing. Ther is a net transfer of isotope from liver to carcass durin this period, which illustrates the sort of difficulty in volved in determining the turnover of protein in th body, considered as a whole. In all probability simila transfers from one protein-synthetic system to anothe occur within individual organs and account to som extent for the apparently non-exponential form of th isotope disappearance.

Tarver and Schmidt (1942) investigated the distribu tion of S-labelled methionine in the proteins of ra tissues one day after administration, and obtained figure for the replacement which are shown in Table 10. I

of particular interest that the intestinal mucosa shows e highest rate of replacement. It is also to be noted at if these figures are to be interpreted in terms of lf-lives' the 'half-life' for intestinal mucosal protein ust be about forty days and those for pancreas and er must be about eighty days.

TABLE 10

e replacement in the dog of protein sulphur by the sulphur of labelled methionine, one day after administration.

Organ or tissue	*Per cent. replacement*
Intestinal mucosa	0·81
Pancreas	0·47
Liver	0·41
Kidney	0·30
Gastric mucosa	0·24
Spleen	0·18
Lungs	0·17
Thyroid	0·15
Brain	0·08
Leg muscle	0·01
Erythrocytes	0·01

These data refer to a single animal which received 10 mg. belled methionine 24 hours before death. On the basis at muscle constitutes about 50 per cent. of body weight d contains 25 per cent. of solids, which is largely protein, d that muscle proteins contain as a rule about 3½ per cent. thionine, the dose of methionine fed is about 0·25 per cent. the methionine content of the animal's skeletal muscle. his serves to indicate what sort of order of replacement could expected.

he concept of the metabolic pool:

hese observations and others like them demonstrate at amino-acids given in the diet pass into the tissues

largely unchanged, and can pass from organ to orga during a protracted sojourn in the body. Schoenheime and his collaborators made much of the point that th nitrogen of administered amino-acids can appear in wide variety of forms of combination in the tissues othe than the one in which it was administered, and it seems t have been on this finding that the concept of a metaboli pool was founded. If the transfer of nitrogen fro amino-acid to amino-acid is an important phenomeno in physiological circumstances, not merely or largely a artefact produced by the feeding of excess of a sing amino-acid, then such a metabolic pool of nitrogen is useful concept. But if, as seems quite probable, th prolonged sojourn of labelled nitrogen in the organis is dependent on a series of sequential transfers, fro intestinal lumen to intestinal mucosa, from mucosa t liver and pancreas, from pancreas back into the lume of the intestine and so round again, from liver to per pheral tissues and plasma, then it may be difficult t locate a 'metabolic pool', however we define it.

For the purpose of further discussion the term mu be defined. It will be used here to describe that part the nitrogen store of the organism which is immediate available to all the tissues capable of nitrogen exchang If we envisage most cells as containing cytoplasm protein in a state of constant flux, and if we suppos that they contribute the breakdown products of suc protein to a common pool, then we must suppose th the major part of body nitrogen stores resides in th cytoplasmic protein, the minor part constituting th metabolic pool. To which part the plasma protein to be allocated is a moot point. If it be available cells in general for ingestion and cytoplasmic synthesi one might suppose that it should be allocated to th metabolic pool. But unless it can be formed and passe into the plasma by all cells it is difficult to allocate the pool without including also the nitrogen of whatev

an or organs transform back the breakdown products cytoplasmic protein into plasma protein. If the sma protein can be destroyed only in the cells of its gin, then it must presumably be regarded, in this text, as a kind of extension of their cytoplasm. This nt will come up again for consideration later.

Clearly, a differentiation of body nitrogen into a abolic pool and a cytoplasmic store is spuriously ple, even if we confine attention to proteins and their nediate precursors and products of breakdown. There at least three well-authenticated instances in the body ypes of cell with life-histories quite other than the arently simple continuities of existence of liver, nerve muscle cells. The instance of the erythrocyte, ch persists in the circulation as a moribund entity some hundred days, with little or no nitrogen exnge between it and its environment, has already been cussed in Chapter II.

Another tissue whose protein metabolism has much same relation to the rest of the organism is the dermis. Here, a deep layer of cells is adequately plied by a blood circulation on its deeper side, and cells of the layer continually grow and divide. The ducts of division are thrust progressively further l further from their sources of nutriment, and prenably because of the peculiarities of their situation, ich provides a progressively diminishing opportunity exchange with the tissue fluids, they die in the racteristic fashion described as keratinization, being n subsequently removed from the body by attrition. this tissue opportunities for exchange in the fashion racteristic of a steady state system are obviously small non-existent.

An instance which raises points of great interest is t of the lymphocyte. It is known that for some cies the daily rate of passage of lymphocytes from the nphatics into the blood circulation is several times as

great as the total number present in the circulation a any one time (Sanders, Florey and Barnes, 1940). The only plausible explanation seems to be that rapid destruction of lymphocytes is constantly occurring Dougherty, Chase and White (1945) have thrown considerable light on this process. In this and previous papers they describe the finding that injections o adrenal cortical steroids into rats previously immunized against a convenient antigen produced simultaneously sharp increase in plasma antibody content and a sharp decrease in lymphocyte count. This led them to examine carefully-washed suspensions of lymphocytes which they found to contain a protein fraction simila in properties to the γ-globulin of blood plasma, i.e. to the plasma globulin fraction in which plasma antibodies are concentrated. The γ-globulin-like fraction of the lymphocytes regularly possesses a higher degree o antibody activity than does the corresponding blood plasma or serum. In connection with these observations others made by Schoenheimer and his colleagues are of interest.

The exchange of ^{15}N between antibody protein and the rest of the organism:

Schoenheimer, Ratner, Rittenberg and Heidelberger (1942) made a study of rabbits actively immunized against a type III *pneumococcus* antigen. The antibody can be precipitated by a specific polysaccharide preparation which is nitrogen-free. Labelled glycine was fed at a time when the total antibody nitrogen concentration in the plasma was decreasing. As Fig. 20 shows the ^{15}N content of the antibody nitrogen rose until the fourth day from the beginning of glycine feeding, and then fell. On the basis that it fell to half the peak value in about two weeks, this time was taken as the half-life of the antibody protein. This sort of estimate should be treated with extreme caution. In the first place, i

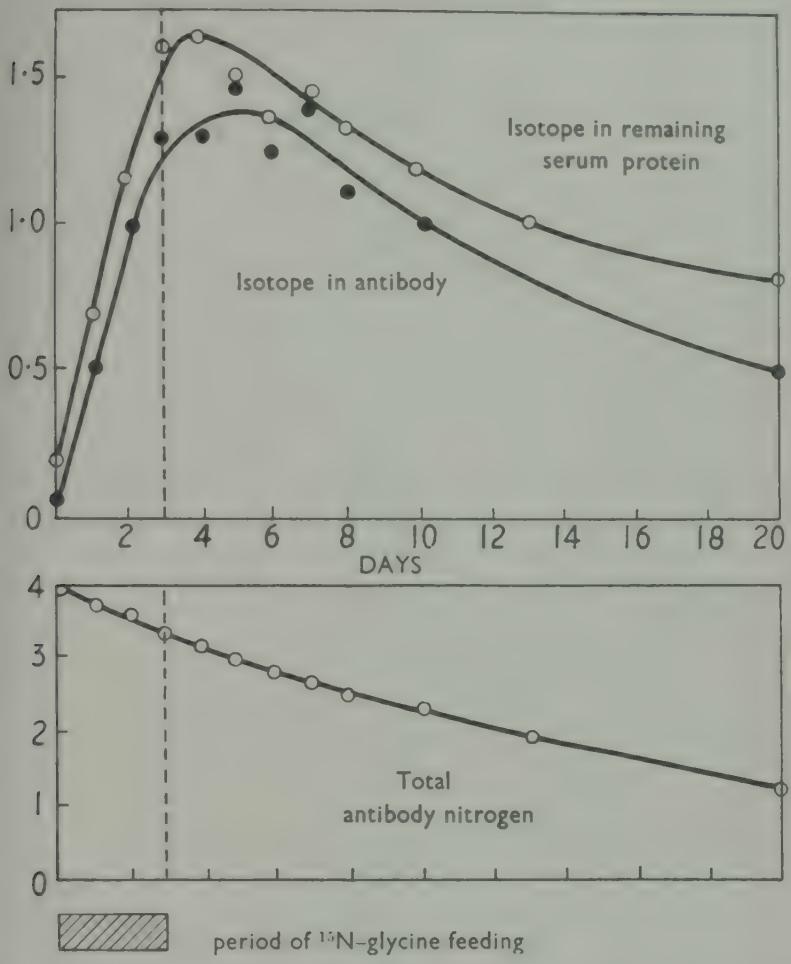


FIG. 20

The degree of labelling of antibody nitrogen and of the maining serum proteins in a rat given ^{15}N-labelled glycine er being actively immunized against type III *pneumococcus* tigen. (*Schoenheimer, Ratner, Rittenberg and Heidelberger* 42.)

ould be noted that the total antibody protein concen- ation in the serum falls from 4 mg./ml. to 2 mg./ml. twelve days, so that there is no question here of a rnover of protein in a steady state system. If we were

to suppose that the rate of destruction of antibod corresponded to one-half in twelve days, then ther would be no way whatsoever of accounting for loss half the proportion of ^{15}N in fourteen days, sinc destruction of antibody results in proportionate los of ^{14}N and ^{15}N. Dilution of isotope requires additio of new protein containing a lower proportion of ^{15}N If, for instance, we were free to assume that about three quarters of the antibody originally present had bee destroyed in the course of fourteen days, and had bee replaced by ^{15}N-free protein equal in amount to quarte the amount of antibody present at the beginning, the we should have accounted for the observations of dis appearance of half the antibody and dilution of its ^{15} to half the original concentration. But the time require for destruction of three-quarters of the protein is tw half-lives (see Fig. 19). If the new antibody forme during the experimental period is not free from ^{15}N will be necessary to postulate an even more rapid ra of destruction of antibody protein and a corresponding shorter half-life.

The observations of Heidelberger *et al.* (1942) in companion paper to the one discussed above show th injected antibody disappears much more rapidly fro the blood than antibody formed by active immunizatio This is in agreement with the necessity indicated abo for explaining the fall in antibody concentration in th serum of the actively immunized animal in terms of destruction more rapid than the observed fall, bu partially offset by continued production. Note th unless the new antibody contributed by this continue production contains very little ^{15}N it is necessary postulate a very short half-life for the serum antibod on the other hand, if the average ^{15}N content of th antibody contributed to the serum, presumably by th lymphocytes, is low enough to be consistent with relatively long half-life of serum antibody, it is necessa

assume that labelled antibody in the lymphocytes, or he lymphocyte containing labelled antibody, has a ery short life. Here, in a physiological context of some omplexity, we have an excellent instance of the neces- ty, which was pointed out by Zilversmit *et al.* (1943), f knowing the time-course of the labelling of the pre- ursor.

Applications of the concept of 'turnover':

So long as attention is confined to a particular class of ell, envisaged as possessing a continuity of existence, ut as dependent for stability of form on continual eplacement of the protein and other nitrogenous con- tituents which are undergoing catabolic transforma- ions, the turnover concept appears to promise some ght on quantitative aspects of protein metabolism. But hen attention is given to the physiological aspects of he matter, the use of the concept appears more difficult. n the instance of the lymphocyte and the antibody it ould appear possible that two turnovers could be pecified, one that of serum antibody, the other that of mphocyte antibody, but this raises no particular ifficulty since presumably, except at times of emergency nvolving introduction of antigen into the organism, here may be expected to be a reasonably steady-state elation between rate of appearance of antibody in the lasma and rate of its disappearance. On the other and, it does not follow that the major site of appearance r disappearance of antibody is in the blood stream, and herefore does not follow that any deductions from erum antibody turnover can be applied to the turnover f the antibody in the organism.

These sorts of considerations invite caution in the nterpretation even of instances in which the set of oncepts 'turnover', 'metabolic pool' and 'half-life' ould appear to be straightforwardly applicable. It is of nterest that Fig. 20 shows very similar time-courses of

disappearance of ^{15}N from plasma protein and from the separated *pneumococcus* antibody. These must represent an accidental similarity, since it is to be presumed that the plasma protein concentration remained constant, whereas that of the antibody certainly did not; the two similar time-courses of disappearance of ^{15}N from the proteins cannot signify similar degrees of replacement.

It has already been pointed out that there are processes of some quantitative importance in protein metabolism, such as the continued formation of epidermal tissue, and the formation of erythrocytes, which do not fit into any picture of acceptance of building-stones from a pool and return of them thither, and, in so far as epidermis is concerned, it is not clear that there is any turnover of cytoplasmic protein at all. Perhaps it should be noted that the 'carcass' to which Fig. 18 refers is composed of muscle, skin and bone, and that Tarver and Schmidt's results (Table 10) suggest that muscle is surprisingly reluctant to take up labelled methionine. It would be of importance to know how much of isotope uptake by 'carcass' was in the nature of irreversible segregation by epidermal tissues, and how much was in the nature of exchange between labile protein and nitrogen 'pool'.

The use of ^{15}N to estimate the overall exchange of nitrogen in the organism:

In view of the complexities discussed in the previous paragraphs, it is of interest to examine in detail the work of Sprinson and Rittenberg (1949), who have analysed the time-course of excretion of a single dose of ^{15}N, administered to man in the form of a very small quantity (10 mg.) of very heavily labelled glycine. The subjects were in nitrogen balance on diets of their own choosing. The time-course of excretion of ^{15}N is followed for three days, and the data obtained are

nterpreted in terms of exchange of nitrogen between a
etabolic pool and the tissues.

Sprinson and Rittenberg fit the empirical equation:

$$F = A(1 - e^{-Bt}) \quad . \quad . \quad . \quad . \quad (i)$$

o the data, where F is the fraction of the administered
otope which has been excreted in the urine between
he beginning of the experiment and the time t, and
A and B are constants. All subsequent interpretations
epend on the acceptance of the values of the constants
f this equation.

Detailed inspection of the data suggests that equation
i) is only a very rough approximation to a description
f them: the time-course of excretion in the published
ull series of data is not at all regular, and the para-
neters of any smooth curve drawn through the set of
oints must be subject to considerable uncertainty. If,
owever, we assume that equation (i) is the appropriate
orm of relation to describe the course of nitrogen
xcretion, then we may pass to Sprinson and Ritten-
erg's physiological interpretation of it.

In order to obtain estimates of protein turnover from
his equation, Sprinson and Rittenberg make the
ollowing assumptions:

(1) that there is one homogeneous metabolic pool containing P g. of nitrogen;

(2) that nitrogen is abstracted from this pool for synthetic processes at a rate of S g. per diem;

(3) that the synthetic systems are in steady states, so that an amount of nitrogen equal to S returns to the pool each day;

(4) that the size of the pool is constant, so that the rates of entry of dietary nitrogen into it, and of loss of waste nitrogen from it, are equal.

(5) that the rate of loss of waste nitrogen from the pool is equal to the total daily nitrogen excretion rate of the subject, which is E g. per diem.

Provided that one further assumption is made, namely:

(6) that the glycine carrying the ^{15}N participates proportionately with other sources of dietary nitrogen in the synthetic activities of the organism, and is converted to waste products at a proportionate rate;

these assumptions lead to simple relations between the empirical constants A and B of equation (i) and the postulated parameters of protein metabolism, i.e. the size of the pool P, the rate of abstraction of nitrogen from the pool for synthesis, S, and the rate of loss from the pool by excretion, E. These relations are:

$$A = E/(E + S) \quad . \quad . \quad . \quad . \quad (ii) \quad \text{and}$$
$$B = (E + S)/P \quad . \quad . \quad . \quad . \quad (iii)$$

These are the forms in which the relations are given by Sprinson and Rittenberg, but for the purpose of further discussion it is more helpful to rewrite them in the forms:

$$S = E\left(\frac{1}{A} - 1\right) \quad . \quad . \quad . \quad . \quad (iv) \quad \text{and}$$

$$P = \frac{E}{AB} \quad . \quad . \quad . \quad . \quad . \quad (v)$$

Putting E equal to the measured rate of nitrogen excretion from the body, which in the ten experiments on moderate diets was in the range $14 \pm 1{\cdot}4$ g. N per diem, S was estimated to be $15{\cdot}5 \pm 4$ g. N per diem and P was estimated to be 36 ± 5 g. N.

The size of the metabolic pool:

Taking these figures at their face value the major point

f importance seems to be the suggested magnitude of ne 'metabolic pool'. If this pool is equally available o all or indeed to any considerable number of tissues, must be distributed by way of the body fluids, and ne striking thing is that if it is supposed to be dis-ributed throughout the body-water uniformly, taking ne water content of the body as 60 per cent., there nust be 80–90 mg. of pool N. per 100 ml. of water. Excluding creatine, carnosine and anserine, as specialized ubstances with local distribution, there is no class of on-protein nitrogenous substance present in the body uids in adequate concentration. After excluding urea rom the non-protein nitrogen of blood plasma, there is ft a total much less than 10 per cent. of the required 0–90 mg. per 100 ml. and much of this must be egarded as outside the pool. A pool of nitrogen in on-protein form, and of the size estimated by Sprinson nd Rittenberg, can only exist if the forms of combination f nitrogen of which it consists are present in the intra-ellular water (constituting some two-thirds of the body-vater) in concentrations averaging one-and-a-half times ne figure of 80–90 mg./100 ml. suggested above. It is ery doubtful if this requirement is fulfilled.

Van Slyke and Meyer (1913–14) and Murray Luck 1928) showed that tissues contain higher concentrations f amino-acids than does plasma, at least as indicated y the relatively non-specific van Slyke nitrous acid nethod. More recently, more specific methods (see Hamilton, 1945) have shown that the α-amino-N of ree amino-acids is present in tissues in higher concen-ration than in blood plasma, and that both contain elatively large amounts of glutamine, the concentration n the tissues again being the larger. There is also some ndication of the presence of small peptides, but not in igh concentration, both in plasma and tissues (Christen-en *et al.*, 1946a and b).

TABLE 11

A tabulation of Hamilton's (1945) data for the amino- plus amide-nitrogen of dog tissues and of Skelton's (1927) data for the weights of dog organs in a form yielding estimates of the probable total amino- plus amide-nitrogen of the whole animal.

Organ	(*N*) *Amino- plus amide-N* (*mg./100 gm. wet*)	(*O*) *Organ- as per cent. body-weight*	(*N*) × (*O*) / 100
Muscle . . .	36·5	43·0	15·70
Intestine . . .	(40·0) (1)	6·5	2·60
Liver . . .	27·3	3·6	0·98
Brain . . .	29·2	1·4	0·41
Lung . . .	16·5	0·9	0·15
Heart . . .	59·1	0·8	0·47
Kidney . . .	25·1	0·7	0·18
Totals . . .		56·9	20·49
Blood (2) . .		7·0	2·55
Cumulative totals .		63·9	23·04
Other soft tissues .	(36·0) (3)	3·1	1·12
Cumulative totals .		67·0	24·16
Skin . . .	(10·0) (4)	16·0	1·60
Skeleton . . .	(10·0) (4)	17·0	1·70
Cumulative totals .		100·0	27·46

(1) This is an average of separate figures given by Hamilton for large and small intestine.

(2) Since Hamilton is not apparently studying blood-free organs it has been taken that the weight of organs would form a larger proportion of the body-weight in his conditions than in Skelton's. Assuming the whole of the blood to lie in these N-rich tissues, the allowance for blood becomes 7·0/56·9 times 20·49.

(3) 'Other soft tissues' are assumed to have the same con-

ntration as those listed earlier: this will be an over-estimate, nce they will consist largely of depot fat.

(4) The estimate of concentration in skin and skeleton is ade as generous as possible: it is thought unlikely that it n be as high as in lung (16·5 mg./100 g.) so 10 mg./100 g. as been taken as well on the generous side.

Accepting the view that the peptide concentration in ssues is low, Hamilton's (1945) data for dog tissues ay be used to give some idea of the content of free mino-N plus amide-N in the whole animal. In Table 11 e column headed *N* gives the amide plus amino-N cording to Hamilton. The column headed *O* gives e organ-weight as per cent. of the body-weight, cording to Skelton (1927). The entry opposite to each

gan in the column headed $\frac{N \times O}{100}$ gives the concentra-

on of amide plus amino-N which would be found in e animal as a whole if the organ in question were the le contributor. Thus if 'other soft tissues' in fact ntributed nothing then the total at the foot of this lumn would overestimate the content of amide plus mino-N by 1·12 mg./100 g. of dog. It seems from is Table that unless some one of the tissues for which uesses have had to be made in the Table contains a ry abnormal amount of amide plus amino-N the animal a whole cannot contain an amount far different from 27 g./Kg., which is about half Sprinson and Ritten-rg's estimate of P (0·51 g./Kg.).

he plasma proteins as a possible metabolic pool:

his makes it difficult to accept any fraction of the non-otein-N as constituting the pool described by Sprinson d Rittenberg. It remains to investigate the possibility at some fraction of the body protein constitutes the ol. The pool cannot consist of intracellular proteins. he pool must be available for exchange, and intra-

cellular protein which can undergo exchange with the rest of the body is obviously a product of synthetic activity applied to materials from the pool. This seems to leave only one source for the pool, namely, the plasma proteins, or a fraction of them.

Sterling (1951) has recently determined the turnover of labelled serum albumin introduced into man, and has incidentally estimated from the initial dilution of this labelled albumin that the total exchangeable pool of serum albumin in the healthy young human male contains in the region of 42 g. N. On this count there is enough to constitute the pool. This figure is very close to Sprinson and Rittenberg's estimate of 30–40 g. N in the pool. Unfortunately Sterling's estimation of the half-life of serum albumin demonstrates that it cannot be identified with the pool. The constant B in equation (i) is the time constant of turnover of the material of the pool, and the half-life of the material of the pool is given by:

$$\text{half-life} = \frac{\log_e 2}{B} = \frac{0{\cdot}693}{B} \quad . \quad . \quad \text{(vi)}$$

Combining this with equation (iii)—

$$\text{half-life} = \frac{0{\cdot}693 \,.\, P}{(E+S)} \quad . \quad . \quad . \quad . \quad \text{(vii)}$$

and substituting Sprinson and Rittenberg's estimates for these quantities in this equation, a half-life for the material of the pool of around 0·8 days is obtained. Sterling's finding for serum albumin is about fifteen days.

Since the heterogeneous collection of proteins which constitute the globulin fraction of serum contain in all only about one-half as much nitrogen as does the albumin fraction, it is unlikely that the pool can be found in this fraction. It seems unlikely that a pool

the size and reactivity of that derived from the prinson and Rittenberg assumptions can exist.

he pattern of metabolic pathways:

efore accepting such an important conclusion it is as ell to scrutinize further the assumptions on which the timate of the size of the pool is based. In the scheme possible pathways shown in Fig. 21 the pathways stulated by Sprinson and Rittenberg are shown as full

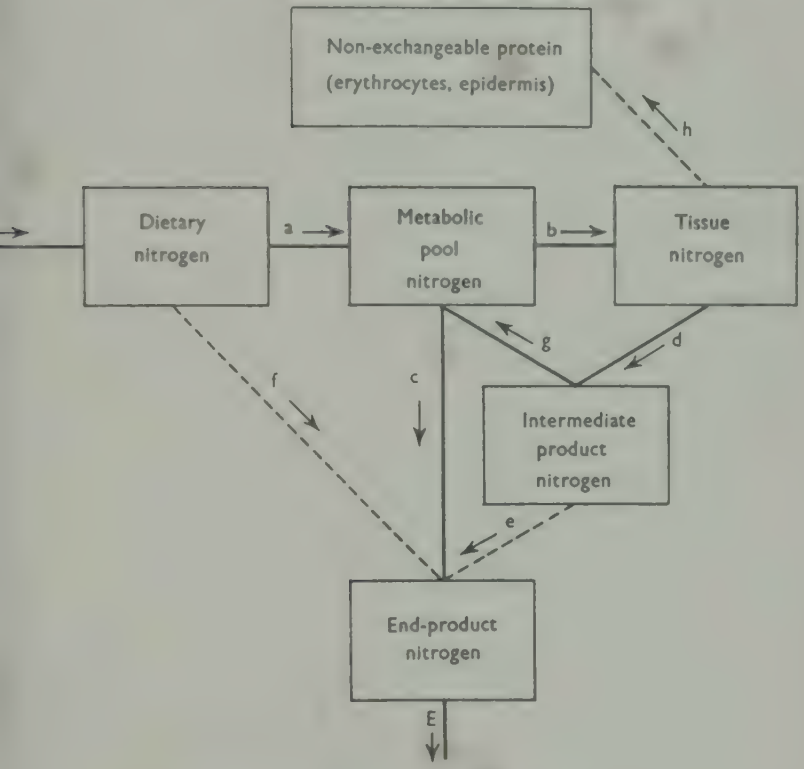


FIG. 21

A scheme of possible pathways of metabolism of ingested trogen to indicate the uncertainty involved in equating the te of urinary nitrogen excretion (E) with the rate of ex-etion of nitrogen from the 'metabolic pool' (c).

lines, with three plausible additional pathways shown as interrupted lines. If in this scheme the net directions of transfer are in the direction of the arrows at velocities symbolized by the letters shown, then it can be shown that if any one of metabolic pool nitrogen, tissue protein nitrogen, intermediate product nitrogen or end-product nitrogen is in a steady state, then all the others are. This is then an internally consistent steady-state system and differs only from Sprinson and Rittenberg's in that entry of new material into the pool is less than D, and that E bears no easily predictable relation either to D or to rate of entry into the pool.

Reference back to equations (iv) and (v) shows that the estimates S and P of rate of use of pool nitrogen for synthesis and of the size of the pool are both directly proportional to the rate of excretion of nitrogen from the pool which is assumed: Sprinson and Rittenberg use E as a measure of the rate of excretion of nitrogen from the pool, but as Fig. 21 indicates, E may be much greater than the rate of loss from the pool. In a more recent treatment of this question Rittenberg (1951) has noted this possibility, and has also revised the treatment so as to confine it to a consideration of urea excretion. In this connection another factor has been introduced, consideration has been given to the effect on the kinetics that results from the fact that there is a 'pool' of urea in the body into which urea passes from the metabolic pool and from which it passes to the urine. The result of this double revision is to reduce the estimated size of the metabolic pool to 2 g. of N in place of the previous estimate of 35 g. In view of this drastic revision, and of the obvious oversimplicities of the model of protein metabolism on which it is based, it seems clear that it is unprofitable to discuss the quantitative aspects of the 'metabolic pool' concept further. So far it has not produced results of much value: it would seem that simple overall descriptions of the time-course of dis-

ɔution of a labelled metabolite are unprofitable in a tem as complex as the mammalian organism, and that ch patience and much study of component systems protein metabolism are needed before the potentiali- of isotopic tracers as agents in the kinetics of tabolism are fulfilled.

nclusion:

e indication of this chapter is that in the study of ermediary metabolism by means of isotopic tracers difficulty of interpretation of the results is at least great as in the older methods of study. It is particu- y important to appreciate the cardinal importance of versmit *et al*'s (1943) condition that the time-course degree of labelling of the immediate precursor of a tabolite must be known if it is desired to study the nover of that metabolite. Now that rare isotopes are ilable in states of high concentration, so that very ensely labelled compounds can be prepared, it is coming possible to use minute doses of test-substances, small to perturb the metabolic pattern of the organ- n. The use of such doses, combined with an increased areness of the complexity of physiological systems, y well lead to great advances in understanding.

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CHAPTER VI

METABOLIC AND ENDOCRINE INTERACTIONS IN PROTEIN METABOLISM

ıe interactions between protein metabolism on the e hand and fat and carbohydrate metabolism on the ıer are superficially simple. It has been known for y years that if either fat or carbohydrate is supplied a starving animal it will reduce the rate of protein abolism, carbohydrate being much the more effective otein-sparer'. Another type of relation between the odstuffs is that it has been suggested on good grounds ıt fat and carbohydrate can be synthesized from the n-nitrogenous moiety of protein. Consideration of e three major foodstuffs on the simple assumptions of erconvertibility, equivalence in catabolism, and beviour according to simple mass action laws leads to nclusions which are not likely to be much in error so far as normal subjects are concerned.

e endocrine factor in metabolic interrelations:

evertheless, it seems that the metabolic relations beeen the major foodstuffs are not governed by simple ıss-action or by isocaloric relations, but by much ore complex endocrine factors. For instance, although rvation normally induces increased nitrogen excretion, ıich is readily explicable on the simple basis of isoric relations, there is no such increase in starved pophysectomized rats (Fisher, Russell and Cori, 36). In order to preserve some such mass action pothesis as that the relative rates of catabolism of the ajor foodstuffs are governed by their competition for

a common enzyme, hydrogen acceptor or oxygen activator, it would be necessary to postulate some step in protein catabolism which occurs only in the presence of a hypophyseal hormone. This type of explanation seems to be excluded by the observation of Soskin, Mirsky, Zimmermann and Crohn (1935) that the fall in blood sugar concentration ordinarily seen in the hypophysectomized dog can be prevented if sufficient protein is included in the diet: it appears therefore that exogenous protein can be metabolized by the hypophysectomized animal. It would seem that the type of relation between carbohydrate metabolism and protein metabolism described above must involve some such sequence as:

1. Diminution in rate of carbohydrate metabolism stimulates secretion of an hypophyseal principle.
2. Hypophyseal principle mobilizes protein from stores.
3. Mobilized protein is catabolized.

Evidence exists for the type of effect suggested under (1). Diminution in rate of carbohydrate metabolism can certainly stimulate the anterior pituitary to secrete at least one hormone: Himsworth and Scott (1938) showed that a hormone directly antagonizing the effect of insulin, i.e. having the properties of the glycotropic factor (Young, 1936, 1938a) is secreted in such circumstances.

Nitrogen retention induced by anterior pituitary extracts. In contrast to the type of effect described in the previous section, which seems to require that there should be an hypophyseal principle stimulating protein catabolism, Teel and Cushing (1930) and Gaebler (1933, 1935) described diminution in nitrogen excretion following

ministration to dogs and cats of extracts of anterior uitary glands.

t now appears that the 'growth hormone' of the erior pituitary is responsible for much of the nitrogen ention activity of these extracts. Li, Simpson and ans (1949) have shown that highly purified prepara- ns of this hormone cause considerable nitrogen ention and that animals treated with these prepara- ns exhibit the same increase in total carcass protein l the same diminution in total carcass fat which had en observed with the cruder extracts. These purified eparations also exhibit the 'diabetogenic' activity t described by Young (1937) and possess as high a betogenic activity as the best previous preparations otes, Reid and Young, 1949).

The nature of diabetogenic activity is that repeated ections of enormous doses of anterior pituitary extract oduce a glycosuria and a ketonuria. The ketonuria idly diminishes and may disappear. If the species animal and its age are favourable, and if the injections sufficiently massive and prolonged, the glycosuria rsists more or less indefinitely after the injections se. The use of the term 'diabetes' for the state so oduced would appear to be misleading, even though ere is in this state what can perfectly justifiably be led a diabetes mellitus. The condition of the animals nevertheless markedly different from what con- utes a state of diabetes mellitus in human medicine. gs rendered permanently glycosuric in the fashion scribed can remain in perfect health for considerable riods without requiring insulin, and with little or no tonuria, despite a massive glycosuria (Young, 1938b; arks and Young, 1939). Such animals appear in- pable of using more than a few per cent. of the calories ingested carbohydrate, but they appear to suffer no advantage from this disability, and they appear pable of utilizing 95 per cent. or more of the calories

of ingested fat, without appreciable increase in ketonuria. This is a state very different from clinical diabetes mellitus.

Metabolic effects of growth hormone:

This principle, facets of whose activity are 'growth hormone' and 'diabetogenic factor' activity, is thus shown to affect the metabolism of fat, carbohydrate and protein. Whether it affects all of them independently, or one of them primarily and the others secondarily is still an open question. Li (1948) has suggested that it exerts a specific stimulant action on protein metabolism, but it is probably much too early to decide whether the action is primarily on protein metabolism, and secondarily on fat and carbohydrate metabolism, primarily on fat metabolism and secondarily on the other processes, or directly and independently on all three of fat, carbohydrate and protein metabolism. What is important is that two patterns of metabolic disturbance now seem to be established, both involving fat, carbohydrate and protein metabolism and both involving the anterior pituitary:

1. The type of disturbance familiar in situations involving tissue carbohydrate deprivation by reason of starvation, failure of insulin supply or paralysis of renal reabsorption of glucose: all these result in increased catabolism of protein and increased fat catabolism and ketosis.

2. The type of disturbance seen in animals given large doses of growth hormone, in which there is increased fat catabolism without there being necessarily a ketosis, and in which there is diminution in the rates of carbohydrate catabolism and of protein catabolism.

It is necessary to leave on one side the question whether this second type of disturbance ever occurs

ysiologically. The answer must wait on the solution such prior problems as the locus of primary action or tions of the growth hormone, and the metabolic ctors, if any, which modify its rate of production by e hypophysis.

Similar points arise in connection with other hypo-nyseal hormones. There are grounds for believing at thyroid preparations and adrenal cortical extracts n influence protein metabolism, and the hypophysis ndoubtedly produces adrenocorticotrophic and thyreo-ophic hormones. It does not seem clear, however, hether the rates of secretion of these hormones are fluenced by alterations in rate of metabolic processes the way in which the rate of glycotropic factor ecretion appears to be influenced. Thus, whilst it is ertain that in so far as the thyroid gland and the drenal cortex may be supposed to set a pattern of rotein metabolism, the hypophysis must be deemed to o likewise, it is not established whether there are rther potential modes of interaction of metabolic rocesses than those already described which are ependent fundamentally on the hypophysis but are ediated by its endocrine dependants.

he influence of adrenal cortical extracts on protein etabolism:

ngel *et al.* (1948, 1949) and Bondy *et al.* (1949) have escribed effects of adrenal cortical extracts on protein etabolism which disclose some further complexities. he work was done by following the changes in blood rea concentration which occur in bilaterally nephrec-omized rats. Injection of adrenal cortical extracts into uch preparations increases the rate of urea formation. dministration of glucose prevents this effect, adminis-ration of an amino-acid mixture fails to increase the ffect, but administration of human serum albumin oes increase it, and this increase is abolished by glucose.

Apart from the fundamental difficulty of all work on adrenal cortical extracts that it is impossible to know the relation between the composition of the extract and the composition of the mixture of hormones secreted into the blood by the adrenal cortex in physiological conditions, there is the trouble that this effect of Engel's is closely bound up with carbohydrate metabolism, which is known to be directly affected by adrenal cortical preparations. It remains of great interest that this work demonstrates that adrenal cortical extracts can stimulate the catabolism of a protein but not of a mixture of amino-acids.

There is some suggestion that the Engel effect is due to a component of adrenal cortical extracts normally capable of secretion into the blood in the work of Hoff (1938), Lewis *et al.* (1940), Swingle *et al.* (1936) and Wells (1940), all of whom have shown that the increase in rate of nitrogen excretion that occurs in phlorrhizin poisoning is much less in adrenalectomized animals than in normal animals. Koepf *et al.* (1941) find that the ability of liver tissue to deaminate amino-acids is as great in adrenalectomized animals as in normals, so that protein catabolism and amino-acid catabolism are again dissociated in this context.

Other endocrine effects on protein metabolism:

It was shown by Janney and Shapiro (1926) very early in the history of insulin that it causes a diminution in the rate of nitrogen excretion in the normal animal. This might be expected if the suggestion were correct that a protein-catabolism-stimulating factor was secreted by the hypophysis in association with the glycotropic factor. Mirsky (1938) has suggested that insulin can directly stimulate the catabolism of administered amino-acids, but his evidence does not seem to require this interpretation.

Kochakian (1937) and Kochakian and Murlin (1935,

936) have produced evidence for an effect of testo-
erone in increasing nitrogen retention in castrated
ogs, and there are indications of complex effects of
estrogens on protein metabolism, which are reviewed
y White (1948). These topics do not warrant an
xtended account in this book. It suffices here to estab-
sh that endocrine factors of considerable complexity
re probably involved in even the simpler aspects of
nteraction of protein metabolism with the metabolism
f fat and carbohydrate.

Gluconeogenesis from protein:

The subject of gluconeogenesis from amino-acids has
een treated at some length and with some scepticism
n an earlier chapter. It is important to realize that
ailure to establish that glucose is formed from a large
xcess of a single amino-acid does not exclude the
ossibility that it is formed from protein. Just as it has
een shown in earlier chapters that the primary anabolic
hases of protein metabolism involve the metabolism of
mixture of amino-acids of a restricted pattern, so now
n this chapter there have been intimations that the
atabolism of protein may differ from that of its con-
tituent amino-acids. There is no *a priori* reason why
protein molecule should have to be hydrolysed to its
onstituent amino-acids as a preliminary to any other
atabolic process. Thus, the conclusive demonstration
f the occurrence of gluconeogenesis from protein need
ot mean that gluconeogenesis from individual amino-
cids plays any significant part in normal metabolism.
It is difficult to find conclusive evidence for gluconeo-
genesis from protein. Two recent reviews of the topic
re in Peters and Van Slyke (1946, p. 141) and Soskin
nd Levine (1946, Chaps. IX and XII). The former of
hese relies on the evidence of the ratio of glucose
dextrose) to nitrogen excreted by pancreatectomized or
hlorrhizinized dogs (the D : N ratio) as evidence for

gluconeogenesis from protein, the latter makes the point that the constancy of the D : N ratio, which is supposed to show that glucose and nitrogen are being derived from a common protein precursor in plausible proportions, does not in fact occur. Soskin and Levine point out that if a pancreatectomized animal maintained on insulin is deprived of insulin and fasted, it is in a state of transition between approximately normal health and death, and that it is difficult to choose an epoch in this transition that can be considered to represent both a steady state and a physiological state. They also demonstrate that when obviously inappropriate epochs in this transition are avoided, the D : N ratio still shows such wide variations that it cannot be said to establish any regular relation between the nitrogen excreted and the glucose excreted. The further consideration may be noted that just as there is a 'pool' of urea in the body, so there must be a 'pool' of glucose (presumably closely related to a 'pool' of glycogen) so that there will be time-lags between any increases in rates of production of glucose and urea and any increases in their rates of excretion. There is no reason why these time-lags should correspond: they will do so only if the general level of the rate of production bears the same relation to the order of size of the 'pool' in both instances. The brief discussion on time-course of excretion of glucose and nitrogen in Chap. IV (p. 83) relates to this topic.

The only direct evidence for gluconeogenesis which Soskin and Levine (loc. cit.) offer is that of gluconeogenesis from amino-acids, which they do not consider to be conclusive. Thus, although there is no doubt that experimental interference can increase more or less simultaneously the rate of nitrogren excretion and the rate of glucose loss from the body, the causal relation between the effects is not established. Long, Katzin and Fry (1940) showed that adrenal cortical extracts increased liver and muscle glycogen in fasting rats as well

the glucose content of the body fluids, at the same me as increasing the rate of excretion of urinary nitro- n. The effect was produced in normal, adrenalectom- ed and hypophysectomized animals. The ratio of crease in total carbohydrate content of the animal to crease in amount of nitrogen excreted was not greater an could have been brought about by conversion of otein to carbohydrate.

In a recent review Long (1952) has pointed out there evidence that adrenaline can cause release of the drenocorticotrophic hormone from the hypophysis and at this can produce effects of the kind just described. hus, any amino-acid which can stimulate the adrenal edulla in the fashion suggested in Chapter IV may be pected to produce a very complex effect on carbo- ydrate metabolism. Two of the primary effects may e expected to be rise in blood sugar concentration and cretion of adrenocorticotrophic hormone, and one of ne later consequences of this second effect may be pected to be increased carbohydrate formation from ormal tissue constituents, possibly from protein. These, course, are merely aspects of a complex set of con- equences. They serve as some dim intimation of the tricacy of the systems that physiologists must have the olhardiness to attempt to understand.

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CHAPTER VII

THE NUTRITIVE VALUE OF PROTEINS

e ultimate fate of protein in the body is to be abolized to carbon dioxide, water and a mixture of cific nitrogenous excretory products of which urea is antitatively the most important in the mammal. In course of this process most of the energy of the tein molecule is made available to the organism, but quantity of energy so supplied is normally less than per cent. of the total energy requirement, so that tein is not to be considered as an important energy-plying food. Protein owes its nutritional importance those amino-acid residues occurring in it which are ded as precursors of the tissue proteins and as cursors of specific essential tissue constituents of low lecular weight: creatine, porphyrins, purines and rimidines may be taken as examples. Since the uirements of different tissues of the body for different ino-acids are undoubtedly different, changes in ysiological state which alter the synthetic require-nts of the tissues must be expected to alter the pattern protein requirements.

ysiological state and protein requirement:

is obviously necessary to differentiate between the tein requirement of a growing animal and that of an ult: the proportions in which the protein absorbed incorporated into different tissues of the body will fer widely in the two instances. Pregnancy, lactation, st-traumatic and post-operative states also constitute nditions of presumably special protein requirement.

In all these states there is the possibility that the lability of the tissue proteins will manifest itself in a competition between tissues for a limiting amino-acid, with some such outcome as that of the experiment of Holt, Albanese *et al.* (1942) already referred to in Chapter IV, in which adult male subjects maintained nitrogenous equilibrium and body weight on an arginine-free diet, but showed a very sharp fall in sperm count. In these experiments the testis clearly did not compete successfully with other tissues for the available arginine. This is one of the few failures of small organs which would appear unlikely to react obviously on growth or health within a moderate period of time.

The general problem of detection of inadequacy of supply of organs of slow turnover remains. We have already alluded to the synthesis of haemoglobin in this connection. If erythrocytes survive in the circulation for 120 days with their haemoglobin intact and inaccessible to catabolic influences, then a diet whose inadequacy reduces the rate of haemoglobin synthesis to 50 per cent of normal will require nearly two weeks to produce a 5 per cent. fall in haemoglobin concentration in the circulating blood, and this fall may well not be detectable as a systematic phenomenon unless large numbers of animals or subjects are employed, together with the most precise analytical methods. This is in spite of the fact that the diet specified would ultimately lower the haemoglobin concentration of the blood to 50 per cent of the normal concentration, a level not usually considered compatible with efficient physiological function. That is, a diet adequate in protein for growth of those tissues having the highest turnover of protein in the body, or in an adult, for the apparent maintenance of nitrogenous equilibrium, may yet be ultimately unsatisfactory by virtue of its failure to nourish tissues in which for one reason or another, deficiency of protein or amino-acid supply is not promptly manifest. This is

sibility that cannot be allowed for in any simple eral method of assessing nutritional value of proteins, nevertheless requires to be kept constantly in d.

ic nature of estimates of nutritional needs:

trition is a practical problem and it is probably true the best way to tackle such a problem is to suppose all estimates of nutritional requirements, however le, should include an allowance for contingencies not luded in the trials or theoretical estimates on which estimates are based. However, two different types nutritional estimate may be required for different pur-es. There is the estimate of requirements of cattle, s, chickens or other farm stock, where environment likely to be reproducible and food consumption form in kind and quantity. In addition, the diet such stock is likely to be required to permit a tricted and reproducible range of physiological ction. In such instances commercial considerations y require reduction of contingency allowances to a nimum by refinement of the estimates of require-nts for a given performance.

The second type of estimate is that which must be d for human requirements, where the range of ivity and environment of any individual is likely to great and where the composition and uniformity of diet will differ widely, and in considerable measure controllably, from individual to individual. No con-gency allowance can be made which can be expected be completely successful: it is only possible to ferentiate protein rations likely to be inadequate in ny circumstances and rations likely to be adequate most circumstances.

Most work on the nutritional value of proteins has en carried out in conditions which provide the first e of estimate: it is important to recognize that such

estimates are not very easily applicable to problems of human nutrition.

Amino-acid composition and nutritional value of proteins:
Major technical difficulties arise out of the number of potentially important nutrient constituents of proteins. Work on the bearing of amino-acid composition on the nutritive value of proteins began in the first decade of the century in the work of Hopkins and of Osborne and Mendel. The discussion of Chapter IV indicates that it is not clear that the number of independently important nutrient constituents of protein is yet known, and that there may be a good case for supposing that pattern of distribution of constituents may be important in addition to number of constituents furnished.

Even if it be accepted as a working hypothesis that proteins provide ten essential nutrients (i.e. Rose's ten essentials) plus two constituents, cystine and tyrosine, capable of sparing some part of the requirement of two of the essential nutrients, there still remain problems of protein nutrition of the growing rat which are technically difficult to solve. Outstanding problems are:

1. Is the requirement of a protein determined largely or wholly by its content of a limiting essential constituent?

2. What are the requirements of the essential constituents, as judged by some stable nutritional criterion?

3. What quantitative criterion of nutritional value can be applied generally?

Biological value of proteins:
A paper by Block and Mitchell (1946) goes a long way toward providing answers to these questions. This paper is a summary of a great volume of work, and marks a step in the study of protein nutrition as important as that made by Rose when he first succeeded in producing

rowth in rats on a diet in which purified amino-acids eplaced protein.

The paper demonstrates the plausibility of the view hat the nutritional value of a protein is determined by s content of a limiting essential amino-acid, when the utritional value is expressed as the biological value of he protein measured in the growing rat. The 'biological alue' is determined in principle in the following nanner. The animal is found to excrete in its urine an mount F of nitrogen when it is fed on a diet adequate n calories but free from protein. When it is given the rotein under test in a quantity providing an amount f nitrogen P, it is found to excrete daily an amount of itrogen E. If the protein fed has no nutritional value is to be expected that the rate of nitrogen excretion ill rise to (F + P). If E is less than this, it is taken hat the protein has 'spared' an amount of body nitrogen qual to (F + P − E). This amount expressed as a per-entage of P, is called the biological value of the protein ed. That is, accepting the interpretation of the observa-ions used in this account, the biological value is the mount of body protein nitrogen spared by 100 parts f nitrogen in the form of the test protein. What has een said in Chapter VI concerning interactions in netabolism will make it appear that the detailed explana-ion of this 'sparing' is probably much more complex han simple substitution, but it can suffice for the resent purpose to note that Block and Mitchell have een able to show that 'biological value' and 'g. gain in ody weight per g. protein absorbed' are linearly related o one another in the rat, so that biological value is robably as good a nutritional criterion as if it did epresent the direct efficiency of sparing of body protein y the dietary protein.

Biological values and chemical scores:

Block and Mitchell have combined Block's extensive

data for the amino-acid composition of proteins with the existing data for biological values, many of them from Mitchell's laboratory, by devising a 'chemical score' for each protein based on the difference between that protein and the mixed proteins of the hen's egg with respect to content of the ten essential amino-acids. Whole egg protein has a biological value of 96 which has been taken as not appreciably different from 100. The chemical score is computed by expressing the content of each essential amino-acid in the protein as a percentage of the content of the same amino-acid in whole egg protein, and taking the lowest percentage found as the score. This then gives the amount of protein of biological value 100 which can be replaced by the protein under examination, in so far as the content of its scarcest amino-acid is concerned.

However, this is a somewhat arbitrary procedure, since it assumes that there is no limiting amino-acid in whole egg protein. It is, of course, possible to conceive of a protein in which there is no limiting essential amino-acid, i.e. one in which all the essential amino-acids are present in exactly the proportion to the total nitrogen to fulfil the animal's needs, but such a protein is not the only one which could have a biological value of 100: there is plenty of room for replacement of a part of the non-essential amino-acids of such a protein by luxus supplies of essential amino-acids.

Modification of the chemical score concept:

Block and Mitchell's results suggest a very strong correlation between content of the limiting amino-acid, expressed as per cent. of the content of the acid in whole egg protein, and the biological value (see Fig. 22). However, the biological value is systematically higher than would be expected if the limiting amino-acid content were the determinant of biological value. It must be supposed either that some other factor than

mino-acid deficiency plays an important part in deter-
ining the nutritional value of protein or that minimal
equirements of the various amino-acids estimated on
ie basis of exact adequacy of whole egg protein are
general excessive. Since this second possibility is the
asier to test, it should be examined first.

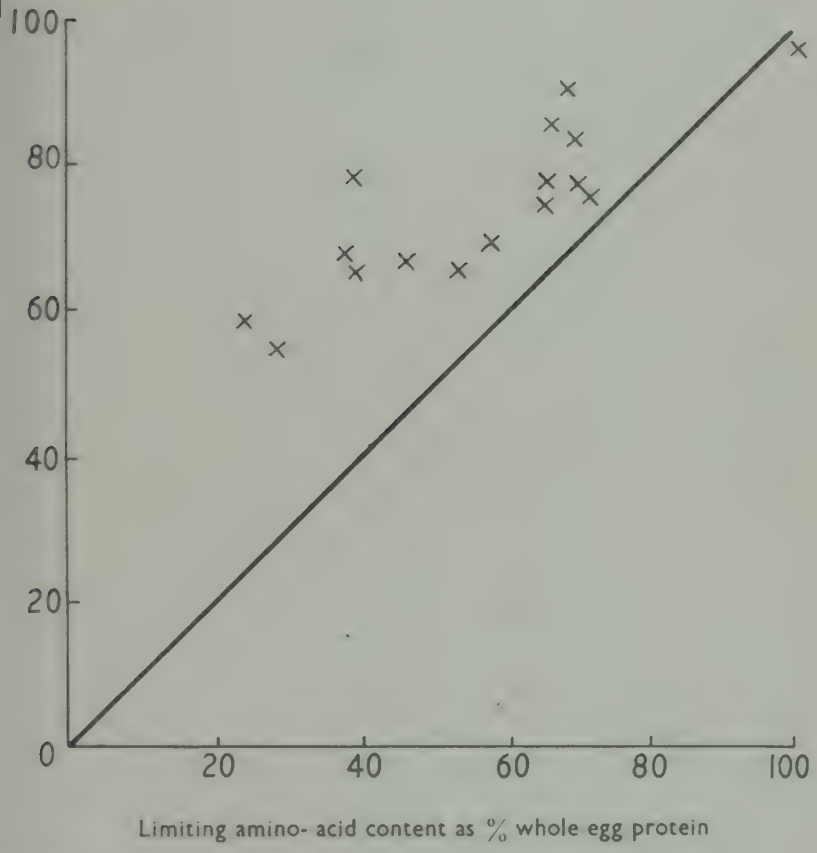

FIG. 22

The relation between biological value for the rat and 'chem-
cal score' (*as defined by Block and Mitchell*, 1946) for the pro-
eins listed in Tables 12 and 13. The line represents the
elation to be expected if biological value were to be equal to
hemical score.

In Block and Mitchell's data there are eighteen protein sources for which complete analyses of essential amino-acids and unequivocal estimates of biological value are available. In Table 12 the data for six of these are presented in the form of 'adjusted essential amino-acid contents'. The 'adjustment' consists in multiplying the content of each amino-acid by (100/biological value). The amount of each amino-acid so obtained is the amount present in a quantity of protein that will exactly substitute for 100 g. of protein of biological value 100. The limiting amino-acid, whatever it is, is then present in each column of Table 12 in an amount corresponding to the amount per 100 g. of protein of biological value 100.

If any of the values so obtained are lower than the corresponding values for whole egg protein they should be indicative of luxus components in whole egg protein, and should themselves be better estimates of limiting requirement than the corresponding figures for whole egg protein.

The lowest estimates in Table 12 are shown in heavy type, and it will be seen that there are six instances in which other proteins give distinctly lower estimates than does whole egg protein. The full series of minimal requirement estimates are collected in the right-hand column of Table 12 under the heading of 'Ideal protein'. A test has been applied to determine whether this term is an overstatement. If this 'ideal protein' is of any general significance, an estimate of its composition derived from a completely different set of proteins should correspond closely with that given in Table 12. The remaining twelve proteins for which Block and Mitchell give full data have therefore been arranged in descending order of biological value and allotted alternately to two groups. The procedure of Table 12 has been applied separately to each group, giving two further independent estimates of the composition of 'ideal protein'. These

TABLE 12

The essential amino-acid contents of representative proteins (as g. per 100 g. protein) scaled-up by multiplying by 100/B.V., where B.V. = biological value. The limiting amino-acid in such scaled-up proteins will be present in just sufficient amount to make the protein of biological value = 100. In the column on the extreme right, headed 'ideal', the lowest figures in each line are collected. Since every entry in the other columns represents not less than the amount of amino-acid present in 100 g. of protein of biological value = 100, the entries in the column headed 'ideal' should be an approximation to the composition of a protein containing just sufficient of every essential amino-acid to have a biological value = 100. All biological values in this Table are true only for the rat.

Source:	*Whole egg*	*Cow's milk*	*White rice*	*Beef heart*	*Casein*	*Peanut*	*Ideal*
B.V.:	**96**	**90**	**75**	**74**	**69**	**58**	**100**
Adjusted content of:							
ARG	6·7	**4·7**	9·6	9·9	5·1	15·8	4·7
HIST	2·2	2·9	**2·0**	3·6	4·4	3·6	2·0
LYS	7·5	8·3	**4·3**	9·9	11·4	5·1	4·3
TRYPT	**1·6**	1·8	1·7	1·9	1·7	1·7	1·6
TYR	**4·7**	5·8	7·5	5·9	10·0	7·5	4·7
PhAL	6·6	6·3	8·9	6·8	8·1	9·1	6·3
SUM	**11·3**	12·1	16·4	12·7	18·1	16·6	11·3
CYST	2·5	1·1	1·9	1·6	**0·4**	2·6	0·4
METH	4·3	3·7	4·5	4·3	**2·0**	5·1	2·0
SUM	6·8	4·8	6·4	5·9	5·5	**4·6**	4·6
THREO	5·1	5·0	5·5	6·3	6·0	**2·6**	2·6
LEUC	**9·6**	12·4	12·0	11·2	11·1	11·9	9·6
isoLEUC	8·3	9·4	7·1	6·9	9·4	**5·1**	5·1
VAL	**7·6**	9·2	8·4	8·4	9·7	11·4	7·6

estimates are presented in Table 13 together with the estimate from Table 12. The very remarkable similarity of the three estimates suggests strongly that the 'ideal protein' has a real significance. There are only two

TABLE 13

Estimates of the composition of a protein having biological value of 100 for growing rats

Amino-acid	*Estimate* 1	*Estimate* 2	*Estimate* 3	*Overall Estimate*
Arginine	4·7	4·6	6·3	4·6
Histidine	2·0	2·2	2·5	2·0
Lysine	4·3	3·7	4·0	3·7
Tryptophan	1·6	1·5	1·5	1·5
Tyrosine	4·7	5·2	?	4·7
Phenylalanine	6·3	6·3	6·4	6·3
SUM	11·3	11·5	10·9	10·9
Cystine	0·4	1·8	1·7	0·4
Methionine	2·0	2·1	2·1	2·0
SUM	4·6	4·4	4·6	4·4
Threonine	2·6	5·1	4·7	2·6
Leucine	9·6	10·4	9·5	9·5
Isoleucine	5·1	5·1	5·4	5·1
Valine	7·6	6·9	6·2	6·2

The first of these estimates is taken directly from Table 3. Estimates 2 and 3 are derived exactly similarly from the data for the following proteins:

Estimate 2		*Estimate* 3	
Protein	*Biological value*	*Protein*	*Biological value*
Lactalbumin	85	Egg albumin	83
Maize germ	78	Beef liver	77
Beef kidney	77	Beef muscle	76
Sesame seed	71	Whole wheat	65
Rolled oats	66	Sunflower seed	65
Whole maize	54	Peas	48

The query in the tyrosine row in estimate 3 is due to the absence of a figure for the tyrosine content of peas.

nstances, those of lysine and valine, in which the later estimates are at all appreciably lower than the first estimates, and the change in estimate in the more extreme instance is less than 20 per cent.

Fig. 23 has been constructed using the overall estimate of ideal protein given in the right-hand column of Table 13. It will be seen that all the proteins, not

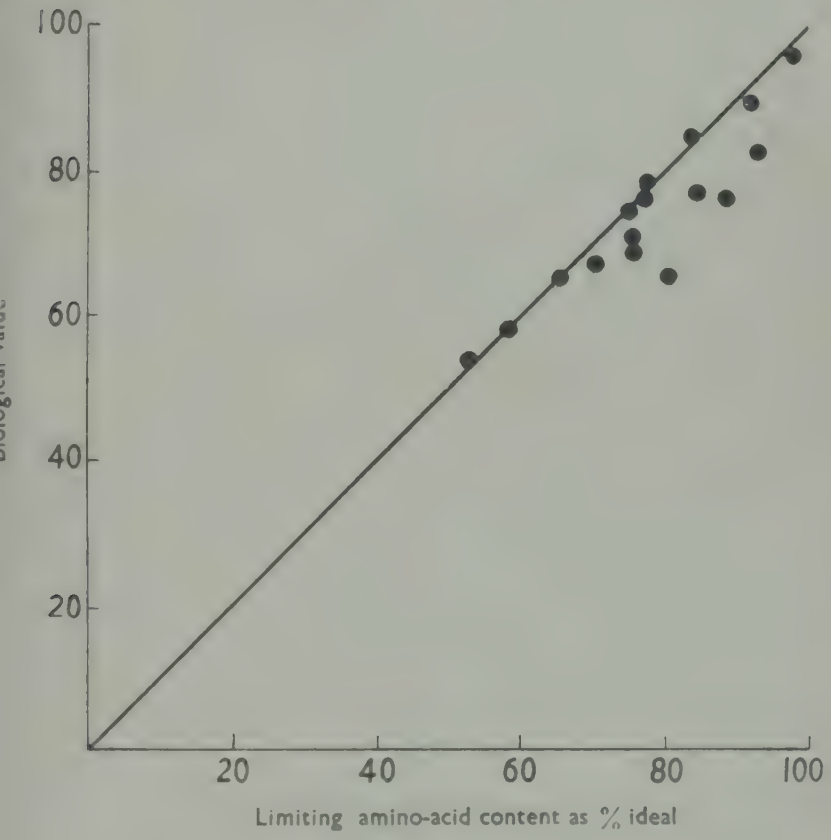

FIG. 23

The relation between biological value for the rat and chemical score based on the 'ideal protein' of Table 13, for the same proteins as those represented in Fig. 22. The line again represents the relation of equality of chemical score and biological value.

merely those which determine the estimate of a particular limiting concentration, agree on this basis in giving a close approximation to equality between biological value and content of limiting essential amino-acid, expressed as per cent. of the content of the ideal protein of Table 13.

Heat treatment and nutritional value of proteins:
Three different sets of proteins give the same answer when they are used to provide an estimate of the composition of protein of biological value 100, on the hypothesis that biological value is directly proportional to content of limiting essential amino-acid, and this lends strong support to the hypothesis. But there are still difficulties in the way of its acceptance. One of these lies in the demonstration that the biological value of a given protein source may be modified by treatment which can hardly be supposed to modify its amino-acid composition significantly. The best known instance is that of soya bean meal, which was first shown by Osborne and Mendel (1917) to be improved in nutritional value by heat treatment. Many subsequent investigations have shown that heating can improve or impair the nutritional value of proteins, but there seem to be no firmly established instances of change of amino-acid composition following heating. Most workers in this field attribute the changes in nutritional value to changes in digestibility. Block and Mitchell (loc. cit.) review this topic thoroughly.

Melnick *et al.* (1946) have shown that proteolytic enzymes liberate methionine more readily from heated than from unheated soya bean meal, though the ultimate extent of methionine liberation is the same from both. If this sort of effect is responsible for the rise in biological value of soya bean meal from 57 to 75 on heating, as reported by Block and Mitchell, then it would appear that there cannot be a simple relation between nutritional

value and amino-acid composition. On the ideal protein basis soya bean meal should have a biological value of 68, which is sufficiently close to the figure for heated soya bean meal to constitute agreement, provided that some special ground can be found for the non-correspondence of the unheated meal. Soya bean meal has been shown to contain a trypsin inhibitor which is thermolabile (Ham and Sandstedt, 1944; Bowman, 1944) and it is likely that this is responsible for some of the peculiarities of this protein. Bowman (loc. cit.) suggested that this might be so, and showed that the navy bean, whose nutritional value is also increased by heat treatment (Johns and Finks, 1920) also contains a thermolabile trypsin inhibitor. Fortunately, instances in which nutritional value is improved by heating appear to be rare, so that effects of this kind may be taken not to require abandonment of the hope of a general simple correspondence between limiting essential amino-acid content and nutritional value.

The biological value of gelatin:

There is, however, still one instance which presents difficulties. This is the demonstration by Mitchell, Beadles and Kruger (1927) that gelatin has a biological value of 25. Unfortunately, gelatin contains no detectable amount of tryptophane and should therefore have a biological value of zero, on the hypothesis of correspondence between content of limiting essential acid and biological value. A possible explanation of this discrepancy may lie in the relation between tryptophan and nicotinamide (see Stokstad and Jukes, 1949). The rat does not ordinarily require the supply of nicotinic acid or nicotinamide as such in the diet: it appears to synthesize its requirements from dietary tryptophan. Feeding protein poor in tryptophan to the rat leads to a fall in rate of excretion of nicotinamide derivatives (Rosen, Huff and Perlzweig, 1946) and administration

of tryptophan increases the rate of excretion of these substances (Perlzweig, Rosen, Levitas and Robinson, 1947). Extra tryptophan also cures the syndrome produced in the pig by nicotinamide deficiency.

Since an animal on a low tryptophan diet develops a nicotinamide deficiency it would appear that the nicotinamide-synthesizing apparatus does not compete successfully for the available tryptophan. If this be so, then nicotinamide deficiency will not be expected to lead to increased protein breakdown. On the other hand, nicotinamide deficiency leads to cessation of growth, which might be supposed to involve depression of protein metabolism. There would seem therefore to be three possible effects of tryptophan lack on protein metabolism: (1) a disturbance of the balance of anabolism and catabolism due to lack of tryptophan for protein synthesis; (2) a small or absent effect on protein catabolism due to demand for tryptophan for nicotinamide synthesis, and (3) a possible depressant effect on protein turnover due to nicotinamide deficiency. If this third effect were large enough in the conditions in which biological value was determined it might account for the apparent biological value of gelatin: feeding a tryptophan-deficient protein might give the appearance of sparing body protein by depressing its catabolism. It would be of great interest to know whether the biological value of gelatin was reduced when nicotinamide was added to the diet.

The significance of the effects of supplementing proteins with single amino-acids:

It has been indicated that two possible difficulties in the way of accepting the limiting amino-acid hypothesis could conceivably be removed. A third remains. Block and Mitchell's form of the hypothesis, leading to the relation illustrated in Fig. 22 suggests that some other factor than content of limiting amino-acid plays an

important part in determining the nutritive value of proteins in general. The modified hypothesis presented here does not require the assumption of a second factor determining nutritive value, but it does require that the limiting amino-acids should be different from those indicated by Block and Mitchell's criterion.

Block and Mitchell present a great deal of data to indicate which are the limiting amino-acids in proteins (loc. cit., Table 12) as determined in experiments in which the protein in question is fed to rats with supplements of different amino-acids: the amino-acid which most readily causes resumption of growth is taken to be the limiting amino-acid. This type of evidence is difficult to accept as confirmatory of the chemical score assessments because inspection of the data shows that there are few, if any, instances of simple deficiency of amino-acids in proteins. The protein of whole wheat may be taken as an instance. According to Block and Mitchell's assessment, i.e. by comparison with whole egg protein, it contains 37 per cent. of the ideal content of lysine, which is therefore given as the limiting essential amino-acid. But, by the same criterion it contains only 45 per cent. of the ideal requirement of isoleucine, so it is difficult to see how the nutritional value of the protein could be improved to any significant extent by supplementation with lysine. Judged by the 'ideal protein' criterion of Table 13 this same protein contains 70 per cent. of ideal content of isoleucine, 71 per cent. of each of leucine and valine and 73 per cent. of lysine. On either of these bases it is difficult to account straightforwardly for the demonstration by Mitchell and Smuts (1932) that whole wheat protein supplemented with lysine supported growth significantly better than whole wheat alone. Corresponding data for whole wheat protein supplemented with the other deficient amino-acids is not available.

In general, both the whole egg criterion and the ideal

protein criterion of Table 13 suggest that proteins are almost equally deficient in a number of amino-acids, so that it does not appear that any useful confirmation or otherwise of chemical methods of assessing nutritional value can be obtained by supplementing proteins with single amino-acids. It would seem desirable that confirmation should be attempted by comparing proteins supplemented with all but one of the suspected deficient amino-acids with proteins supplemented with all suspected deficient amino-acids.

Nutritional value and biological value:

In view of the difficulties inherent in the physiological interpretation of 'biological value' it may seem disproportionate to spend so much time in attempts to relate it to the chemical composition of proteins. But Block and Mitchell have shown that biological value is related simply to the ability of the protein to support growth. Fig. 24, which is redrawn from their paper, shows that there is a regular relation between g. gain in body weight per g. protein consumed and the biological value of the protein. It is not to be expected that the one would be in direct proportion to the other, since it is to be presumed that there will be maintenance requirements that must be met before there can be surplus for the laying-down of fresh tissue. The scatter of the observations around a uniform progression may be taken as due in unknown proportions to the variability of the experimental conditions in respects other than protein ration, to the necessary uncertainty in the precise value of the experimental estimate of biological value, and to real differences between proteins of the same biological value but different amino-acid composition in their ability to support growth. It would seem that such differences cannot be very large, so that if the relation between biological value and amino-acid composition can be substantiated, the relation between amino-acid com-

position and ability to support growth can also be taken to be substantiated.

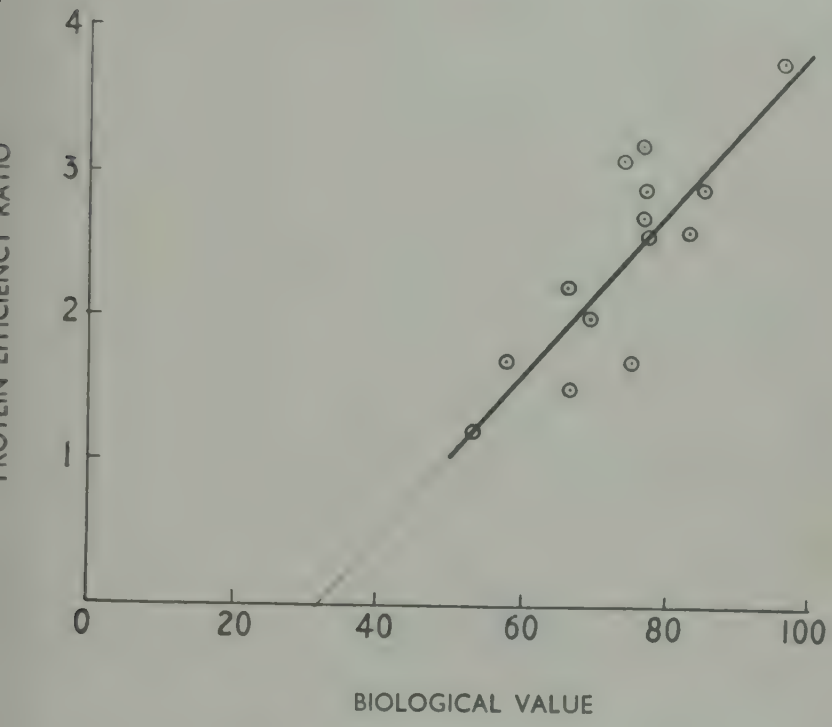

FIG. 24

The relation between the 'protein efficiency ratio' in the rat (g. gain in body weight per g. protein absorbed) and biological value for the fourteen proteins of Figs. 22 and 23. (*Data of Block and Mitchell*, 1946.)

Supplementary relations between proteins:

If proteins could be ranked in order of nutritive value on the basis of amino-acid composition then it would become possible to predict the extent to which proteins can supplement one another. This question of supplementary relations is of the greatest practical importance, but has hitherto been approachable only by the most

laborious trial and error. If proteins are dependent for their nutritive value on their content of some one or more amino-acids present in limiting concentrations, then the nature of the supplementary relations are unpredictable without knowledge of the concentrations of all essential amino-acids in the protein. The nature of the relations to be expected is best illustrated by a model.

TABLE 14

Model proteins, to illustrate supplementary relations between proteins, based on the distribution of amino-acids in them

Amino-acid	*Content of amino-acids in the model proteins expressed as per cent. of the animal's daily requirement present in* 100 *g. of the protein*			
	Protein 1	*Protein* 2	*Protein* 3	*Protein* 4
A	60	200	120	180
B	**20**	180	100	**20**
C	40	100	80	120
D	120	**40**	180	100
E	180	200	**40**	40

In Table 14 it is supposed, for ease of exposition, that proteins contain only five essential constituents, denoted by the letters A to E. The amounts of these constituents are expressed as the percentage of the animal's daily requirements which are to be found in 100 g. of protein. The constituent present in the lowest relative concentration will determine the biological value of the protein. The units used are such that the concentration of the limiting constituent is numerically equal to the biological value. These limiting concentrations are shown in heavy type in Table 14. It will

noted that all four model proteins shown are of low ological value, proteins 1 and 4 each having a biological lue of 20, whilst 2 and 3 have biological values of 40.

TABLE 15

ne composition of mixtures of the model proteins described in Table 14

Amino-acid	*Mixtures of equal parts of proteins*				
	(1+2)	(2+3)	(3+4)	(1+4)	(1+2+3+4)
A	130	160	150	120	180
B	100	140	60	**20**	**80**
C	**70**	**90**	100	80	85
D	80	110	140	110	110
E	190	120	**40**	110	110

Since mixtures of equal parts of two or more of these roteins will have compositions which are the arithmetic eans of those of the components it is easy to explore ie effects of mixing. Table 15 presents some typical ata. Mixture of equal parts of proteins 1 and 2 yields 'first class' protein of biological value 70. Mixture of roteins 2 and 3 yields an exceptionally good protein f biological value 90. On the other hand, mixture of roteins 3 and 4 yields a product whose biological value no higher than that of the better component. The ixture of the two proteins 1 and 4 each of biological alue 20 yields a product of biological value 20. Mixture f all four in equal proportions yields a product of high iological value. The amount of body protein spared by 00 g. of this mixture is computed to be 80 g., whereas the 25 g. contributions of the individual proteins were ed separately they would spare in all a total of 30 g. of

body protein. This may appear a naïve approach to the estimation of the effectiveness of supplementation, but it is unlikely to underestimate the degree of effectiveness: reference to Fig. 24 suggests that proteins of biological value less than 40 will not support growth, and in these models none of the component proteins has a biological value of over 40, whereas three of the simple mixtures have biological values well over this lower limit.

'First-class' and 'second-class' protein:

The sort of relation shown in Fig. 24 accounts for the tendency to classify proteins into first-class proteins capable of supporting growth and second-class ones not capable of doing so. But if supplementary relations occur between proteins in nutrition this classification is without practical value. Very few practical diets contain a single protein or a single source of protein. Further, the discussion of the model of supplementation in the last section suggests that second-class proteins in combination may be in effect first-class, so that the distinction is often a misleading one. The particular importance of the large amount of work on the relation between the amino-acid, acid composition and biological value of individual, proteins is that it appears unlikely that any other approach can provide an answer to the prime practical problem of specifying the nutritive value of the proteins of a normal mixed diet.

Species differences in protein requirement:

Block and Mitchell (loc. cit.) note that the whole egg criterion does not give good guidance concerning the nutritive values of different proteins for chicks. Among other factors determining this difference may be expected to be the requirement of the chick for glycine as an essential amino-acid. Quite apart from this, however, there seems to be no very good reason why the quantitative requirements for different amino-acids should be

in the same proportions for different species. It is known that arginine is on the border-line as an amino-acid essential for growth in the rat, whereas it is absolutely necessary for growth in the chick. The discussion of the preceding few sections of this chapter must be taken to refer strictly to the rat. Similar studies would now seem to be well worth while for other species, but the conclusions to be drawn from studies of the rat ought not yet to be extended even in the most general form to other species.

It should be remembered that experimental work with amino-acid mixtures has suggested that (i) in some species a number of amino-acids other than Rose's essentials may be necessary for optimal growth and (ii) that any considerable excess of some amino-acids in a mixture may reduce the nutritive value of the mixture, despite the fact that the proportion of each essential amino-acid in the mixture remains at or above the optimum. In the rat it would seem that neither of these factors plays a great part in determining the nutritive value of a protein. This may merely mean (i) that the biological value criterion does not differentiate readily between proteins giving good growth and proteins giving optimal growth, and (ii) that proteins in general do not contain grossly excessive proportions of individual amino-acids as judged by the requirements of the rat. The fact that these points do not have to be taken into account as of first importance in determining the practical value of protein in nutrition does not mean that they are not of importance in the theory of protein metabolism.

Conclusion:

The great volume of work done on the nutritional value of protein to the rat has been brought together by Block and Mitchell into a statement of the relation between amino-acid composition and nutritive value

which appears to offer the prospect, both for this species and for others, of specifying the nutritive value of the majority of proteins on the basis of their chemical composition alone. Exceptions such as soya bean meal and gelatin can occur and still call for some complete explanation, but it seems unlikely that they can be frequent enough to detract from the usefulness of this approach.

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CONCLUSION

In the review of a major topic of this kind, two chief sorts of approach are possible. The writer may record the conclusions of workers in the field and may connect them together in the fashion which they appeared to these workers themselves to fit together, or he may attempt what is more truly a 're-view', an interpretation of the discovered facts, as distinct from the published hypotheses, in relation to as many as possible of the other facts which have subsequently accumulated. This second kind of approach, which is attempted in this book, is bound to be imperfectly effected, particularly because of the immense inherent difficulty of comprehending a large body of factual material without the aid of some sort of working hypothesis, which almost certainly becomes a source of prejudice: the major pleasure of scientific research is in the creation of successful working hypotheses, but the affection their authors tend to conceive for such creations can be dangerous: like Galatea, the hypothesis comes to have a life of its own.

However, this is a difficulty of the ordering of the results of any scientific research, and particularly so of those of biological research, where the systems studied are of such complexity that very few first principles can be said to have been discerned. The biologist must, in gross, conform to the fundamental principles of the exact sciences, but otherwise there is little restraint on the form his hypotheses need to take, and it is often difficult to determine whether two hypotheses constructed to account for different aspects of related biological activities are consistent or no. As a consequence,

hypotheses which initially direct inquiry into profitable channels may survive after they have ceased to do so, and hypotheses which fail to account for subsequently established relations between phenomena may persist.

The general interpretation adopted in the book is that implicit in the notion of 'continuing metabolism', namely that synthetic processes, mainly those leading to cytoplasmic protein synthesis, are the immediate fate of the products of absorption of protein into the body. The only novelty in the present approach is the emphasis placed on the incompatibility of this interpretation with the notion that the major processes of protein metabolism can be profitably studied by investigating the fate in the organism of individual amino-acids. The historical accident of the early emphasis on the importance of the free amino-acids in protein metabolism has meant that in parts of this book an attitude has had to be adopted that might be stigmatized as hypercritical. The position is adopted in Chapter I, for instance, that there is no firm demonstration that the products of protein absorption pass into the body either exclusively or chiefly as amino-acids. It is pointed out that all the evidence is indirect and none of it conclusive. The reader may very fairly object that the difficulties of chemical physiology are such that much the same qualifications hedge round most of its generalizations. This is undoubtedly true, but it means no more than that the mammalian organism, the object of the major attentions of chemical physiology, is very highly complex and that most simple generalizations concerning it must be crude approximations, subject in the course of time, it is to be hoped, to gradual refinement. These refinements will arise only where some agency undermines previous confidence in a hypothesis. In the writer's view it is better that the agency should be a reasonably systematic one: dramatic accidents do occur which upset long-cherished hypo-

theses, but they do not occur often enough to warrant the leaving to them of the direction of the course of theory. On the other hand, accident always plays a large part in determining the course of theory, and greater awareness of the part it plays might help us to a greater degree of detachment.

The following passage is taken from *Problems in Animal Metabolism*, by J. B. Leathes (1906):

"The situation may be summed up in a few words. Till recently, it was believed that proteins were absorbed mainly if not entirely as albumoses and peptones; that these substances were converted by a synthetic change carried out in the intestinal mucous membrane into the coagulable proteids found in the blood; that these blood proteids supplied the needs of the body, and were the material used for all tissue repair. We have gradually learnt that the first of these articles of belief requires considerable modification, we have to recognize that the second remains purely hypothetical, and that, therefore, the third is little if anything more than a preconception.

"The problem as it presents itself to us now is rather this: is the synthesis of proteid, which is so important a factor in the metabolism of all growing and living animals, a function only of the intestinal epithelium? Direct evidence is not as yet forthcoming, and we must be content with some working hypothesis put together from general physiological and biological considerations. But the result of attempting to form such a hypothesis at the present time will be somewhat different from what it was a few years ago. We know that in the seeds of plants proteids are often stored in considerable quantity as foodstuff for the growing seedling. During germination these proteids are hydrolysed, and circulated in the sap in the form of the familiar cleavage products. Schultze and Winterstein have isolated from the seedlings of various species of

plants a long list of these cleavage products: leucine, iso-leucone, amido-valerianic acid, alanine, glutamic acid, aspartic acid, phenyl-alanine, tyrosine, pyrrolydine carboxylic acid, cystine, tryptophane, lysine, arginine, histidine. All these substances can be formed during digestion in animals. In the plant it is in these forms that the nitrogenous material is supplied to the cells during the period of most active growth, and from these unquestionably the proteids are synthesized. In animals until recently we have believed that the intestine synthesized from these or more complex substances the serum-albumin and globulin found in the blood, and that it was with these highly organized coagulable proteids that the cells of the body were actually nourished. No account has been commonly taken of the fact that these proteids of the blood must be taken to pieces and again put together, rearranged on a different plan, if they are to serve for the making of proteids and nucleo-proteids in the cells of the muscles and other organs in which the destructive changes of life are felt. The proteids circulating in the blood are a currency which is not legal tender' (pp. 141–2).

It was against this background that Van Slyke showed with his nitrous acid method that there is a rise in the amino-nitrogen content of deproteinized blood taken during digestion and absorption of protein. It is not surprising that opinion swung from the already doubtful view that blood proteins were the 'legal tender' of protein metabolism to the other extreme at which the free amino-acids were to be regarded as having this function. In the years that have elapsed since Van Slyke's discovery the advance of physiology into a realm of what is at least semi-exactitude has given us grounds for doubting the certainty with which changes in blood concentrations were sometimes interpreted in the past. We can now sometimes add to all the other questions

we put to nature, the question, is the change big enough or small enough to support the hypothesis we are testing? It is when we begin to try to apply this sort of test to the notion of the free amino-acids as legal tender in protein metabolism that we begin to see the difficulties of the hypothesis.

There is a great deal to be done before any reasonable degree of certainty can attach to any hypothesis concerning the nature of the forms of circulating material involved in the exchanges of protein and amino-acid derivatives in the mammalian organism, and nothing in this book precludes the possibility that it will ultimately prove to be the case that the circulating material is entirely in the form of free amino-acids. But it is worth noting that if the products of absorption of protein by a particular species consisted of as few as five pentapeptides, each, we may suppose, capable of combining with any of the other four, then a protein molecule of 200 amino-acid residues, which is not large for a protein molecule, could be constructed from these five peptides in 10^{12} different ways. If we were to postulate scission of some of the intermediary products at points other than those of condensation of pentapeptide residues, and recombination in different order, the number of possible different products would be increased and the pattern of amino-acid sequence in them would be obscured. At the same time, if the postulated pentapeptides were the products of activity of the animal's intestinal mucosa, there might be sufficient of a pattern of amino-acid distribution in the proteins to account for the overlaying of a species specificity on the local characteristics of the individual proteins. This is pure speculation, and the only justification for introducing it here is that it embodies the notion that if peptides play a part in protein metabolism, it may well be in the form of specific peptides: it does not follow that if some peptides are not metabolized by some mammals, no peptides are

metabolized by any mammal. If the theme of this book is in any large degree justified, that the metabolism of protein is the metabolism of the amino-acids in concert, amino-acids must come together in some form, and short of a miraculous conjunction in a reaction of the hundredth order there must be a series of intermediates of great importance, which must fall into this category of the neglected peptides.

Many reviews have been made of aspects of protein metabolism. The following list is a selection designed to cover a wide range of topics and of points of view. It does not include reviews which have already been referred to earlier in this book.

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INDEX